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CONTINUED DEVELOPMENT AND TESTING OF A SUSTAINED RELEASE SYSTEM--ETC(U)
APR 78 J D GRESSER, D L WISE DAMD17-74-C-4120
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DYNATECH R/D COMPANY

CONTINUED DEVELOPMENT AND TESTING
OF A SUSTAINED RELEASE SYSTEM
FOR THE PREVENTION OF MALARIA

Annual Report

April 1978

(for the period 24 December 1977 - 23 March 1978)

by

J. D. Gresser, Ph.D.
D. L. Wise, Ph.D.

Supported by

US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-74-C-4120

DYNATECH R/D COMPANY
A Division of Dynatech Corporation
Cambridge, Massachusetts 02139

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Matrices each containing 50 w/w% of ^{3}H-WR-7557 or ^{14}C-WR-158122 in 90L+/10G copolymers of 220,000 and 49,000 respectively have been prepared. The former has been molded into 1.5 mm diameter beads, the latter cryogenically ground and sieved to 45-180μ particle size. A third matrix is being prepared containing 50 w/w% acedapsone in the 49,000 molecular weight copolymer used for ^{14}C-WR-158122.

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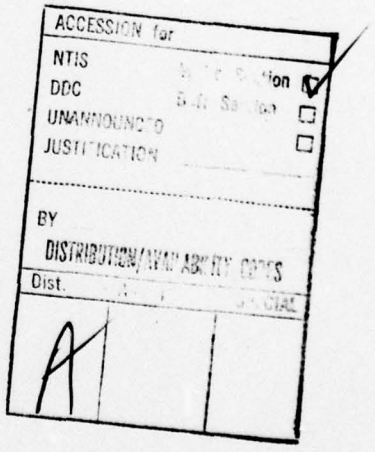
The matrices will be used as components of two dual drug systems. System I is to include WR-7557 and WR-158122 in a 10/1 wt. ratio. System II is to include DADDS and WR-158122 in a 10/1 wt. ratio.

Three baboons will receive System I with a one additional baboon serving as a control to receive a 10/1 mixture of the pure drugs. Two baboons will receive System II with two additional serving as controls. All baboons are to receive a total drug dose of 50 mg/kg.



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Section 1

SUMMARY

The purpose of this phase of Dynatech's program with Walter Reed Army Institute of Research is to develop and test two dual drug systems for malaria prophylaxis. The systems to be tested are as described in the following table.

Table 1.1

Summary Description of Dual Drug Systems

System	I		II	
Drugs	WR-158122	WR-7557	WR-158122	DADDS
Drug Label	C-14	H-3	C-14	H-3
Polymer Compositions	90L+/10G	90L+/10G	90L+/10G	90L+/10G
Polymer Molecular Weight	49,000	220,000	49,000	49,000
Drug Loading in Matrix (percent by wt. of drug)	50.0	50.0	50.0	50.0
Parts by Weight of Each Matrix	1	10	1	10
Dosage Form	45-180μ injectable powder	1.5 mm diameter beads	45-180μ injectable powder	45-180μ injectable powder

Both systems are to consist of two matrices. System I is now complete and contains both sulfadiazine (WR-7557) and 2,4-diamino-6-(2-naphthylsulfonyl)quinazoline (WR-158122). The former, tritium labeled, has been incorporated at a loading of 50% by weight into a 90L+/10G copolymer of 220,000 molecular weight. The latter, carbon-14 labeled, has been incorporated also at 50 wt.% into a 90L+/10G copolymer of 49,000 molecular weight. Synthesis and characteristics of both polymers have been described in Dynatech Report No. 1711 (WRA-5), Quarterly Progress Report No. 14.

System II is to contain the carbon-14 labeled WR-158122 matrix described above as well as a matrix containing tritium labeled acedapsone (DADDS). The synthesis of DADDS has been completed by Amersham Corporation and the matrix is currently being fabricated in our laboratories. The DADDS matrix will also contain 50% by weight of the drug in the low molecular weight polymer being used for WR-158122.

Construction of the two dual drug system involves the preparation of three matrices, one for each drug. The sulfadiazine matrix has been molded into 1.5 mm diameter beads for subcutaneous implantation. The WR-158122 matrix is to be injected and has therefore been cryogenically ground and sieved to retain the 45-180μ range of particle sizes. The acedapsone is also to be ground sieved to 45-180μ particle size.

The plan is to inject/implant these materials into baboons at the University of Alabama Medical School as indicated in Table 1.2.

Table 1.2

Summary Description of Baboon Test Program

System	I	II
Total Number of Baboons	4	4
Baboons Receiving Matrices	3	2
Baboons Receiving Pure Drug Mixture (no polymer)	1	2

Baboons will be injected/implanted with 50 mg/kg body weight of total drug, not including polymer, with 1 part WR-158122 to 10 parts of either WR-7557 or DADDS. Testing of baboons will be conducted over a three month period by measuring carbon-14 and tritium in urine and feces.

During this program year two manuscripts have been prepared and submitted for review. These are included in this report as Appendices B and C.

Section 2

DESCRIPTION OF DUAL DRUG SYSTEMS

2.1 System I: WR-7557 and WR-158122

System I consists of two drugs, WR-7557 and WR-158122 separately incorporated into polymer excipients. The WR-7557, tritium labeled, is incorporated into a 90L+/10G polymer of molecular weight 222,000 at a loading of 50% by weight to give a matrix specific activity of 540 μCi/gram. The matrix has been molded into beads of 1.5 mm diameter suitable for implantation.

The WR-158122, carbon-14 labeled, has been incorporated into a polymer of similar composition, 90L+/10G, but with a molecular weight of 49,000, also at a loading of 50% by weight. The carbon-14 specific activity of this matrix is 192 μCi/gram. The particle size of this matrix has been reduced to 45-180μ by cryogenic grinding.

These matrices are to be delivered to the test animals in a weight ratio of 10 parts WR-7557 to 1 part WR-158122.

Serving as controls will be a 10/1 mixture of these drugs delivered without a polymer excipient.

2.2 System II: Acedapsone (DADDS) and WR-158122

System II consists of DADDS, tritium labeled, and WR-158122 carbon-14 labeled as above. The two drugs are to be simultaneously delivered in a 10/1 weight ratio as mixed matrices, with controls of a pure drug mixture. Both drugs will be present at 50 wt. % loading in the 90L+/10G polymer described previously with a molecular weight of 49,000. The WR-158122 matrix is complete; the DADDS matrix is currently being fabricated.

Tables 2.1 and 2.2 present data on the WR-7557 and WR-158122 matrices.

Table 2.1

DESCRIPTION OF WR-7557/POLYMER MATRIX

Composition:		
Wt. WR-7557		5.5785g
Wt. Polymer 30026-b		5.5807g
Wt. % WR-7557 in Matrix		49.99%
Activity of Matrix		540μCi/g
Residual Solven by GC	Benzene	0.18%
	THF	0.61%
Polymer Description:		
Composition of 30026-b		90L+/10G
Molecular Weight		$\overline{M}w$ = 220,000

Dose Form: 1.5 mm diameter beads

Table 2.2

DESCRIPTION OF WR-158122/POLYMER MATRIX

Composition:		
Wt. WR-158122		1.2894g
Wt. Polymer 27970		1.2894g
Wt. % WR-158122 in Matrix		50.00%
Activities of Matrix		192 μCi/g
Residual Solvent by GC	p-dioxane	0.13%
Polymer Description:		
Composition of 27970		90L+/10G
Molecular Weight		$\bar{M}w$ = 49,000

Dose Form: 45-180μ powder

Section 3

IN VIVO EVALUATION OF DUAL DRUG SYSTEMS

Both systems are to be evaluated in baboons. Table 3.1 summarizes plans for these experiments. The dual drug system consisting of WR-7557/WR-158122 will be given to 4 baboons, three of which will receive the drugs in matrix form, and one serving as a control will receive a mixture of the pure drug. The ratio of WR-7557/WR-158122 is to be 10/1 on a weight basis with a total drug dose of 50 mg/kg of body wt. This means that each animal will receive 45 mg/kg of 7557 and 4.5 mg/kg WR-158122. As each animal weighs about 15 kg, the total dose of WR-7557 will be ∿675 mg (1350 mg matrix ≃450 beads). The wt. of WR-158122 is planned to be 67.5 mg or 135 mg matrix.

The second system, WR-158122/DADDS, will be delivered to two baboons, with two more serving as controls to receive the pure drug mixture. Here again the wt. ratio of DADDS/WR-158122 will be 10/1 with a total drug dose of 50 mg/kg body wt.

A protocol for delivering the drug and for sample collection is presented in Appendix A.

Table 3.1

BABOON INJECTION SCHEDULE

A. System WR-158122/WR-7557

Drug	Dosage Form	Label	Parts by Weight Drug into Animal
WR-158122	90 - 180 μ powder	^{14}C	1
WR-7557	1.5 mm dia. beads	^{3}H	10

NOTE: 3 baboons to receive polymer/drug matrix, 1 baboon to receive pure drug mixture - all @ 50 mg drug/kg animal weight.

B. System WR-158122/DADDS

Drug	Dosage Form	Label	Parts by Weight Drug into Animal
WR-158122	90 - 180 μ powder	^{14}C	1
DADDS	90 - 180 μ powder	^{3}H	10

NOTE: 2 baboons to receive polymer/drug matrix, 2 baboons to receive pure drug mixture - all @ 50 mg drug/kg animal weight.

Section 4

CREDIT

The authors wish to acknowledge the able assistance of Mr. Steven Crooker, Ms. Letha Chemmalil, Mr. Robert Cheyne, and Ms. Jane Knowles in conducting the polymer syntheses, GPC measurements, fabrication of the matrices, and activity measurements.

Appendix A

TENTATIVE PROTOCOL FOR EVALUATION OF DUAL DRUG SYSTEMS IN BABOONS

Appendix A

TENTATIVE PROTOCOL FOR EVALUATION OF
DUAL DRUG SYSTEMS IN BABOONS

A. Introduction

Two dual drug systems are to be evaluated in baboons with respect to the rates at which each drug or its metabolites are excreted in urine or feces.

System I consists of WR-7557 (tritium labeled) and WR-158122 (carbon-14 labeled) each separately incorporated into a polymeric excipient at 50 wt. % loading. The WR-7557 matrix is formed into 1.5 mm beads; the WR-158122 matrix is ground and sieved to 45-180 particle size. The two drugs are to be delivered in a weight ratio of ten parts WR-7557 to one part WR-158122. Controls will be a mixture of the pure drugs (no excipient) in the same weight ratio.

System II consists of acedapsone (DADDS) and WR-158122 each separately incorporated into a polymeric excipient at 50 wt. % loading. Both matrices are ground and sieved to a particle size of 45 - 180μ. The powdered matrices are blended to contain 10 parts by weight of DADDS and one part WR-158122. Controls will be a mixture of the pure drugs in the same weight ratio.

Each dual system is to be delivered at a total pure drug dose of 50 mg/kg body weight.

Matrix specifications are as follows:

	WR-7557 Matrix	WR-158122 Matrix	DADDS Matrix
Excipient Composition	90L+/10G	90L+/10G	90L+/10G
Excipient Mol. Wt.	220,000	49,000	49,000
Wt. % Drug in Matrix	50 Wt. %	50 Wt. %	50 Wt. %
Matrix Form	1.5mm Dia. Beads	45-180μ powder	45-180μ powder
^{14}C-activity of Matrix	---	192 μCi/g	---
^{3}H-activity of Matrix	540 μCi/g	---	---

B. Shipment of Dual Drug Systems

System I (WR-7557 and WR-158122):

(a) Twelve vials will be supplied each containing ∿338 mg of the WR-7557/polymer matrix in bead form. This will be approximately 113 beads per vial.

(b) Three vials will be supplied each containing 135 mg of the WR-158122/polymer matrix.

(c) One vial will be supplied containing a mixture of 675 mg of ^{3}H-WR-7557 and 67.5 mg of ^{14}C-WR-158122. This is to serve as the control.

System II (DADDS and WR-158122):

(a) Four vials will be supplied each containing a mixture of 675 mg of DADDS/polymer matrix and 67.5 mg of WR-158122/ matrix.

(b) Two vials will be supplied each containing a mixture of 675 mg of ^{3}H-DADDS and 67.5 mg of ^{14}C-WR-158122. These are to serve as controls.

C. Implantation/Injection Schedule

System I (WR-7557) and WR-158122):

Each of three female baboons is to receive by subcutaneous implant the entire contents of four vials containing the WR-7557/polymer beads. In addition each of these baboons is to receive by intramuscular injection the entire contents of one vial containing the WR-158122/polymer matrix.

Furthermore a fourth female baboon is to receive the entire contents of the vial containing the pure drug mixture which is to serve as the control.

System II (DADDS and WR-158122):

Each of two baboons is to receive by intramuscular injection the entire contents of two vials containing the mixture of the DADDS and WR-158122 matrices.

In addition each of two other female baboons is to receive the entire contents of one vial containing the mixture of pure ^{3}H-DADDS and ^{14}C-WR-158122 which is to serve as a control.

D. Implantation Protocol for the WR-7557/Polymer Beads

1. Anesthetize three female baboons.

2. Shave scapular region on back where incisions are to be made (see Step 3).

3. Make four small incisions parallel to the median line, two anteriorly and two posteriorly, approximately five centimenters apart in each direction using sterile technique.

4. Introduce all beads of WR-7557/polymer matrix contained in a vial into one incision, so that ∿1350 mg (∿450 beads) are delivered to each animal (∿338 mg or ∿112 beads to each incision). The beads should be distributed throughout the subcutaneous area.

5. Each incision should be closed using 4-0 silk suture which should be removed about one week later.

6. Place each baboon in a separate metabolism cage supplied with food and water ad libitum.

E. Injection Protocol for Powdered Matrices and Pure Drugs

Materials for injection are listed below with volumes suspending vehicle (1% w/v methocel) to be added to each vial.

Material	System	Mg. Wt. Per Vial	Ml. Added To Each Vial
WR-158122/polymer matrix	I	135	0.5
WR-158122/WR-7557 control	I	742.5	1.5
WR-158122/DADDS/polymer matrix	II	742.5	1.5
WR-158122/DADDS control	II	742.5	1.5

F. Injection Protocol for WR-158122/Polymer Matrix (System I)

1. Shave injection site on gastrocnemius muscle of each of the three female baboons injected with WR-158122/polymer matrix while the animals are still anesthetized.

2. Add 0.5 ml of 1% w/v methocel to each of the three vials containing the WR-158122/polymer matrix.

3. Suspend particles uniformly by vibrating for 15 seconds on a Vortex-Genie vibrator or equivalent.

4. Withdraw suspension via an 1" x 18 Stubbs gauge hypodermic needle into a 1.0 ml capacity syringe. Insure suspension by repeated withdrawal and discharge from the syringe until uniform consistency is apparent. Use a different needle and syringe for each animal.

NOTE: Some residue will remain in each vial. It is important to account for this material. See Step 7.

5. Insert the needle into the gastrocnemius muscle of each baboon as deeply as it will go. Inject all of the suspension. Withdraw carefully to minimize leak back at injection site.

6. Examine each needle, syringe, injection site and environs for residual undelivered matrix. It is important to account for all material in order to close a material balance. Any spillage or leak back at the injection site is to be wiped with a pledget of gauze wet with dioxane (see Step 7) and added to the dioxane indicated in Step 7.

7. Place each set of syringe, needle, supply vial, and sponge pledget containing undelivered drug/polymer suspension in a known volume (approximately 100 ml) of scintillation grade dioxane (supplied by Dynatech). The purpose is to extract the residual matter in order to determine how much was not injected into the animal. A preliminary wash-through of the needle and syringe by filling and emptying with dioxane is recommended prior to breaking the syringe and needle apart before placed in the dioxane. Send Dynatech 10-20 ml labeled with the baboon identification (I.D.) to determine the amount of undelivered matrix and thus, the amount of matrix delivered to the animal.

4. As in Step F.3.

5. As in Step F.4.

6. As in Step F.5.

7-10. As in Steps F.6-F.9.

I. Injection Protocol for DADDS/WR-158122 Control (System II)

1. As in Step H.1.

2. As in Step G.2.

3. Add 1.5 ml of 1 w/v% methocel to each of the two vials containing the control mixture.

4, 5. As in Steps F.3 and F.4.

6. As in Steps F.5.

7-10. As in Steps F.6-F.9.

J. Schedule for Fecal and Urine Collections

Days -6 to 0	Collect urine and feces daily for combination into a one week pool. These samples will serve as a control for background counts.
Day 0	Implant after daily collection.
Days 1 - 14	Collect urine and feces daily for shipment to Dynatech.
Days 15 - 91	Collect urine and feces daily. Combine each week's pool.

K. Fecal and Urine Collections

Days -6 to 0 - Feces[1]: Collect feces daily and weigh to nearest tenth of a gram. Store samples in freezer until all samples have been collected. Thaw and combine into common pool. Blend in an Osterizer or Stomacher until sample is homogeneous. Remove a 10 - 20 gram sample and place in a scintillation vial[2] with appropriate animal identification and date[3] for shipment to Dynatech[4].

Days -6 to 0 - Urine: Collect urine daily after collecting feces. Rinse cages and pans with no more than 250 ml of water and add to urine. Record total volume of liquid (urine plus rinse) to nearest ml. Mix thoroughly. Store samples in freezer until week's collection is complete. Combine the week's collection, mix thoroughly and send Dynatech a 10 - 20 ml aliquot.

Days 1 through 14 - Feces: Collect feces daily and weight to nearest tenth of a gram. Homogenize each daily sample and send a 10 - 20 gram sample to Dynatech.

Days 1 through 14 - Urine: Collect urine daily, record to nearest ml, rinsing cage with a minimum volume of water. Send 10 - 20 ml aliquot to Dynatech in scintillation vial.

Weeks 3 through 13 - Urine and Feces: Urine and feces are to be collected on a daily basis and combined into a weekly pool as described for days -6 to 0. Homogenization and aliquotting are also to be as for days -6 to 0.

8. Place each baboon in a separate metabolism cage.

9. Collect urine and feces daily.

G. Injection Protocol for WR-158122/WR-7557 Control (System I)

1. Anesthetize or suitably restrain one female baboon.

2. Shave injection site on gastrocnemius muscle.

3. Add 1.5 ml of 1 w/v% methocel to the vial containing the control mixture.

4. As in Step F.3.

5. As in Step F.4.

6. As in Step F.5.

7. As in Step F.6.

8. As in Step F.7.

9. As in Step F.8.

10. As in Step F.9.

H. Injection Protocol for DADDS/WR-158122/Polymer Matrices (System II)

1. Anesthetize or suitably restrain two female baboons.

2. As in Step G.2.

3. Add 1.5 ml of 1 w/v% to each of the two vials containing the mixed matrices.

NOTES:

1. Days -6 through 0 feces and urine will serve as a background count.

2. Scintillation vials should be Fisher brand Linear Polyethylene vials, 20 ml capacity with 27 mm dia. screw caps (Cat. 337-11 or 12) or equivalent. The straight walls of these vials greatly facilitate sample removal. Make sure caps are screwed down tightly and secured with tape.

3. All sample vials and vial caps should be identified with baboon identification (name or number), dates of collection and days post injection. Identification and date should be printed with indelible ink or typewritten on gummed labels which will adhere to vials in event of thawing during shipment.

4. All materials should be stored frozen until shipment and shipped over dry ice.

Appendix B

SUSTAINED RELEASE OF SULFADIAZINE

D. L. Wise, G. J. McCormick, G. P. Willet,
L. C. Anderson, and J. F. Howes

SUSTAINED RELEASE OF SULFADIAZINE

Donald L. Wise, Ph.D.†, Gerald J. McCormick, Ph.D.*
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SUMMARY

Three implantable preparations were evaluated in mice as controlled delivery systems for releasing sulfadiazine (WR-7557) as an antimalarial drug. One preparation, 1.5 mm dia. spherical beads, containing 10.0% sulfadiazine in a copolymer of L(+)-lactic acid and glycolic acid (90% and 10% by weight, respectively), was efficacious against Plasmodium berghei at doses of 66 and 132 mg drug/kg body weight, but the release in mice was non-uniform. A second preparation, also 1.5 mm dia. spherical beads, containing 33.3% sulfadiazine in a copolymer of L(+)-lactic acid and dl-lactic acid (50% each by weight), was efficacious at doses of 58 mg/kg and greater, and the release in mice was uniform. The third preparation, 90 - 180μ cryogenically ground powder, containing 20.0% sulfadiazine in a polymer of L(+)-lactic acid, exhibited little efficacy at doses of 80 and 160 mg/kg and the release was non-uniform. In vivo and in vitro testing of release rates, largely employing ^{35}S-labeled sulfadiazine, was carried out with these samples, and the results were found to be largely supportive of the P. berghei challenge testing. In addition, in vitro release rates of representative systems were measured as a function of drug loading in the matrix and as a function of polymer molecular weight. Also, one system, 50dl/50L(+) with 33.3% WR-7557, was evaluated in vivo using 0.75 mm dia. cylindrical rods and 90 - 180μ cryogenically ground powder in addition to the 1.5 mm dia. spherical beads. Overall, there was negligible evidence of bio-incompatibility.

Successful sustained release of the antimalarial drug 2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline (WR 158122) was reported earlier, using a delivery system with a copolymer of dl-lactic acid and glycolic acid as the implantable vehicle (Wise, McCormick, et al, 1976). In mice, release took place over a 14-week study and protection against infection by the rodent malaria Plasmodium berghei was observed through the same period of time. The object of the present study was the investigation of this sustained delivery system concept for another effective antimalarial drug, sulfadiazine. The system was tested in mice for duration of release and for effectiveness against P. berghei.

MATERIALS

Polymer Preparation

Glycolide and lactide, synthesized from glycolic acid, dl-lactic acid, and L(+)-lactic acid, were polymerized by previously reported procedures (Wise, McCormick et al, 1976; Schwope, 1975). The polymer preparations were: (A) a copolymer, poly[L(+)-lactic-co-glycolic acid], 90% L(+)-lactic acid and 10% glycolic acid [termed here 90L(+)/10G]; (B) a copolymer, poly [L(+)-lactic-co-dl-lactic acid], containing 50% L(+)-lactic acid and 50% dl-lactic acid [termed here 50dl/50L(+)]; and (C) a polymer, poly[L(+)-lactic acid], containing 100% L(+)-lactic acid [termed here 100L(+)]. All compositions are reported in percent by weight.

Antimalarial Drug

Sulfadiazine (WR-7557) was supplied by the Division of Medicinal Chemistry, Walter Reed Army Institute of Research. The compound was also used containing ^{35}S-labeled sulfadiazine in trace quantity (synthesized by Amersham/Searle Corporation, Arlington Heights, Illinois).

Sample Preparation

The 90L(+)/10G preparation, containing 10.0% sulfadiazine, was prepared as molded spherical beads of 1.5 mm dia., each weighing 3.3 mg (0.33 mg sulfadiazine). The 50dl/50L(+) preparation, containing 33.3% sulfadiazine, was also prepared as molded beads of 1.5 mm dia., each weighing 3.39 mg (1.13 mg sulfadiazine). The 100L(+) preparation, containing 20.0% sulfadiazine, was prepared as a 90 - 180μ cryogenically ground powder (Anderson, et al, 1976). For the 50dl/50L(+) system, extruded cylinders 0.75 mm dia. were prepared, as were 90 - 180μ particles. Other selected samples used in screening tests are described in the text but follow earlier reported work (Wise, McCormick, et al, 1976; Schwope, 1975; Anderson, 1976).

METHODS

P. berghei Challenge

Testing for duration of efficacy in vivo was done at Walter Reed Army Institute of Research following procedures previously described (Wise, McCormick, et al, 1976). Preparations in bead form were introduced beneath the skin in the scapular region by means of a 10-gauge needle inserted through a remote incision and manipulated into position. The beads were pushed completely through the needle, which was then withdrawn. The incision was closed by suture. Implantations of 1, 2, 4, and 8 beads were made. The 90 L(+)/10G preparation was implanted in experimental groups of four mice each. The dosages were 17, 33, 66 and 132 mg sulfadiazine per kg body weight. The 50dl/50L(+) preparation was implanted in experimental groups of three mice each. The dosages were 57, 114, 228 and 456 mg sulfadiazine per kg body weight. The 100L(+) preparation, in powder form, was suspended in 0.7% (w/v) carboxymethyl cellulose and injected subcutaneously through an 18-gauge needle into the scapular region. In two studies, experimental groups were of three mice each; dosages were 10, 20, 40, 80 and 160 mg sulfadiazine per kg body weight. Immediately after administration of the delivery system, each animal

received an intraperitoneal injection of P. berghei infected mouse blood (2×10^7 parasitized cells). P. berghei (NYU-2 strain) was maintained by serial passages in mice. Observation of appearance and survival was done daily except on weekends. At seven day intervals, blood smears were taken and examined for parasites. Each animal with a negative smear then received an injection of P. berghei-infected blood, as above. Control groups of five animals were injected in a similar manner each time.

In conducting the research described in this paper, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Animal Resources, National Academy of Sciences-National Research Council.

In Vivo Release

Testing was carried out at SISA, Inc., Cambridge, MA, under the direction of Dr. John F. Howes, Director of Pharmaceutical Research. Male albino CD-1 mice weighing an average of 20 grams were administered rods, beads, or powder containing ^{35}S-labeled WR-7557 subcutaneously in the scapular region. The rods or beads were implanted using a trochar; the powder was injected in 1.0% methyl cellulose. Each mouse was placed in an individual metabolism cage and the urine collected semi-weekly for the first four weeks and weekly thereafter. The amount of ^{35}S-labeled WR-7557 excreted in the urine was determined, using liquid scintillation techniques (the testing of the unlabeled WR-7557 in the 90L(+)/10G beads is described separately, later). Feces were not regularly collected because WR-7557 release is essentially 100% in the urine. At the termination of the experiment, the mice were sacrificed. The residue of injected material was noted and the site examined visually for signs of encapsulation or tissue irritation. Residual WR-7557 at the injection site was determined for matrices prepared as rods or beads. These were removed from the mice, dissolved in scintillation grade dioxane, and the radioactivity measured via liquid scintillation. Residual WR-7557 for matrices prepared as powders was not determined.

In Vitro Release

The in vitro experiments were carried out at Dynatech by placing duplicate amounts of approximately 50 mg each of the rods, beads, or powder (the 90 - 180μ powder of the 50 dl/50L(+) system was not evaluated in vitro) in a Whatman extraction thimble suspended by a thin wire into a test tube containing 65 ml of pH 7 phosphate buffer solution. The test tube was placed in a rack in an oil bath at 37°C and under gentle agitation. Aliquot samples were taken daily at first and approximately weekly for the remainder of the release period. The buffer solution was changed when samples were taken. For samples with ^{35}S-labeled drug, the amount of ^{35}S-labeled WR-7557 leached into the buffer solution was measured, using liquid scintillation techniques. For the 90L(+)/10G beads, spectrophotometric techniques were employed.

RESULTS

Following the logic of the planned experiments, as well as the actual results, some selected in vitro experiments will be described first. Then, animal tests will be presented, supported by in vitro testing results. Due to the manner of screening the samples, results of P. berghei challenge testing are reported at the end of this section.

In Vitro Results: Effect of Drug Loading

Figure 1 depicts the cumulative percent WR-7557 released from the 90 - 180μ powder of the 100L(+) system containing 10.0, 16.7, 20.0, and 33.3% drug. In addition to the steady release of WR-7557, it is interesting to note that the greater the percent of WR-7557 in the sample, the faster the release of WR-7557 in terms of percent of the original implant drug content. In other words, the greater the percent WR-7557 in the sample, the shorter the duration of WR-7557 release. Powders containing 33.3% drug released the material too rapidly for further consideration, while the powders containing less than 20% drug released it too slowly. Powders containing 20.0% drug appeared to achieve WR-7557 release over a three-month period, with

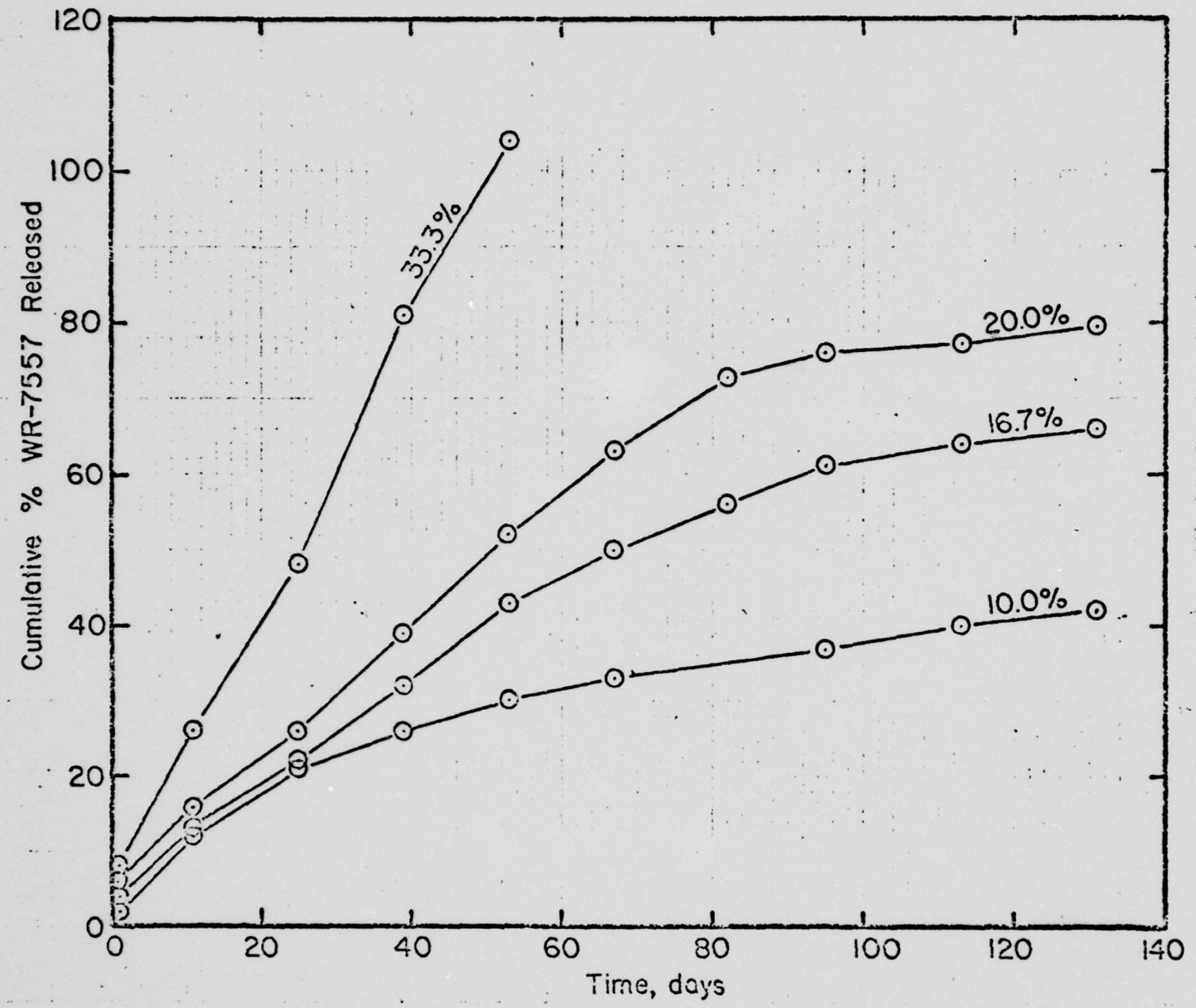

Figure 1

Cumulative Percent WR-7557 Released In Vitro from 90 - 180μ Powders of the 100L(+) System Containing 10.0, 16.7, 20.0, and 33.3% WR-7557

depletion of the drug reservoir shortly thereafter. On this basis, samples of this latter material were used for further study.

In Vitro Results: Effect of Polymer Molecular Weight

The *in vitro* leaching characteristics of WR-7557 from 1.5 mm dia. beads containing 33.3% WR-7557 in 50dl/50L(+) copolymers with molecular weights of 150,000, 210,000, and 450,000 were evaluated. The release patterns observed with these three lactic acid copolymers at three selected molecular weights are presented in Figure 2. After 92 days, the 150,000 MW copolymer had released an average of 80% of the WR-7557 initially present in the sample, the 210,000 released 55%, and the 450,000 released 47%. Thus, the molecular weight of the copolymer appears to be a strong factor in controlling the rate of WR-7557 release, higher molecular weight copolymers resulting in slower drug release. However, each copolymer tested, regardless of molecular weight, released the WR-7557 at a relatively constant rate. The 150,000 MW copolymer released the WR-7557 at a rate most representative of a 90-day system. On this basis, beads prepared using the 150,000 MW copolymer were submitted to WRAIR for *P. berghei* challenge and to SISA, Inc. for measurement of the urinary excretion of WR-7557 *in vivo* from mice.

In Vivo and In Vitro Results for Sample Preparation of 90L(+)/10G @ 10% WR-7557

The *in vivo* and *in vitro* results are illustrated in Figure 3 for beads of the 90L(+)/10G system containing 10% WR-7557. For *in vivo* testing, eight 1.5 mm dia. beads were implanted subcutaneously in the scapular region of four mice. Urine samples were analyzed twice weekly for WR-7557 using the colorimetric method of Bratton and Marshall (1939). *In vitro* testing was as described above. The cumulative percent WR-7557 released *in vitro* and *in vivo* in the urine of the four mice is shown. Since essentially all of the WR-7557 is released in the urine, urinary release is equivalent to total *in vivo* release. After 18 days, the *in vivo* samples had released 12.3% of the WR-7557 initially present in the sample, while after 19 days the *in vitro* sample had released 11.3%. Thus, there appears to be a one-to-one correlation

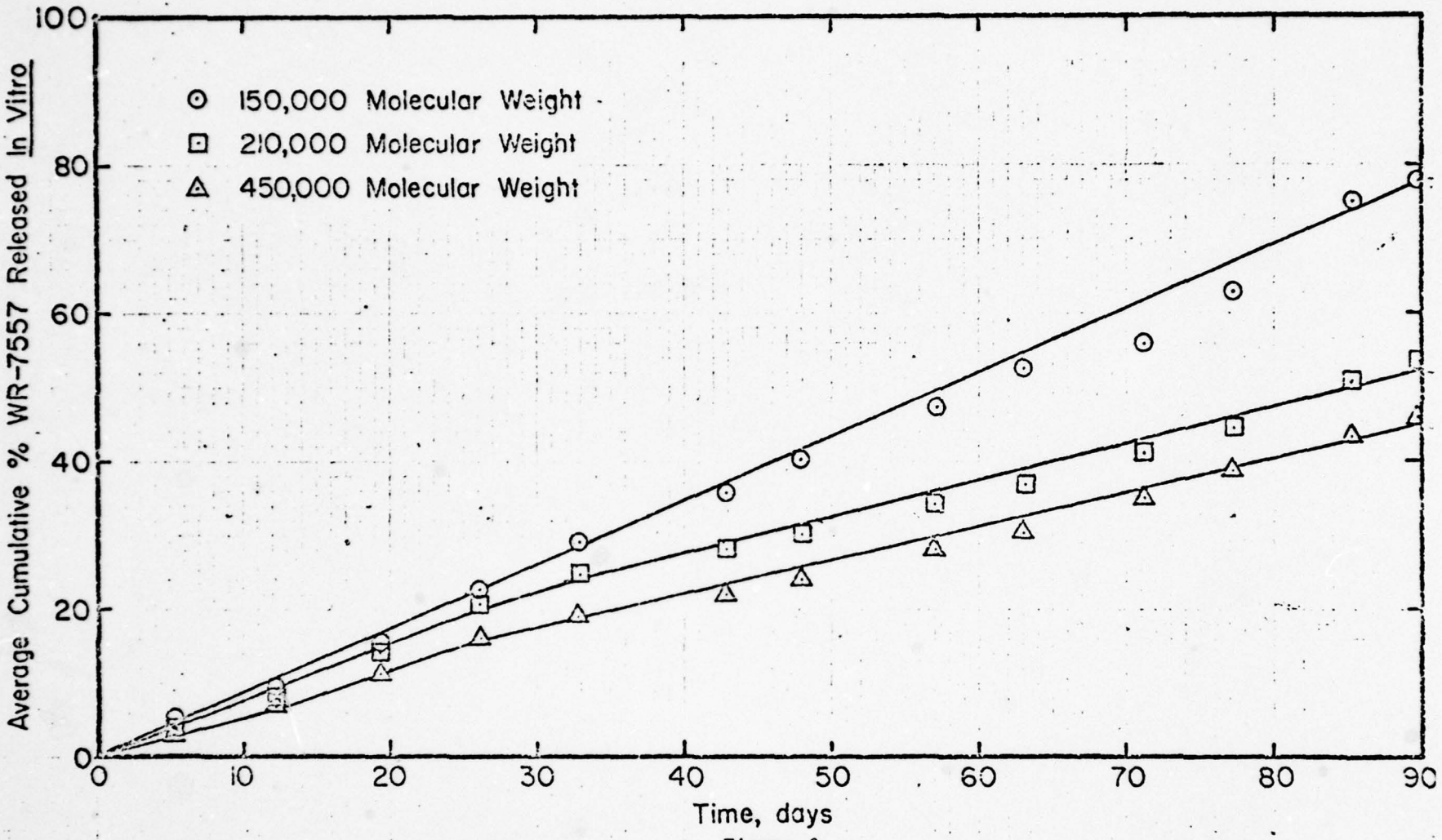

Figure 2

Effect of Molecular Weight of 50dl/50L(+)-Lactic Acid Copolymers on the Cumulative Percent WR-7557 Released In Vitro from 1.5 mm Diameter Beads Containing 33.3% WR-7557

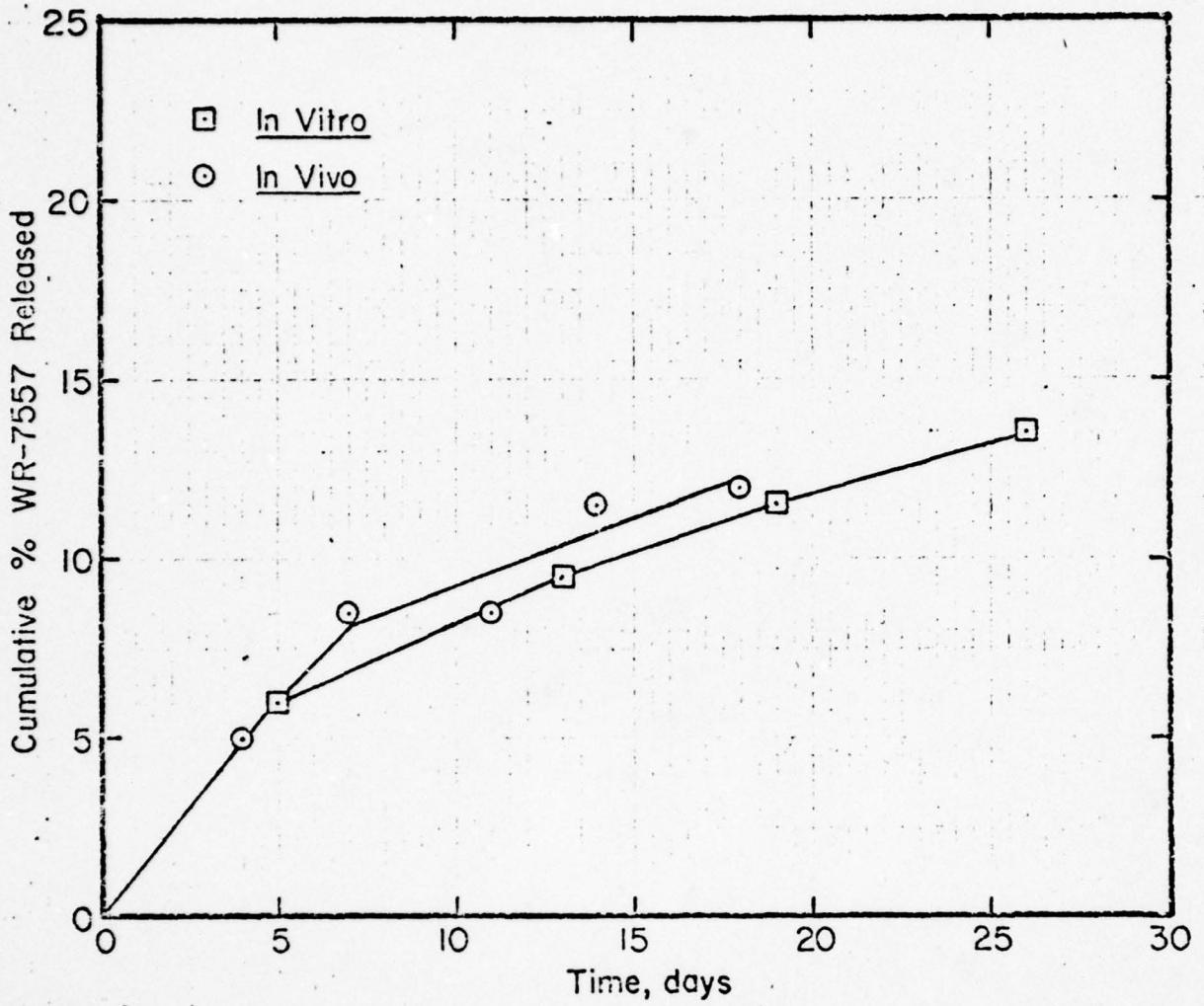

Figure 3

Cumulative Percent WR-7557 Released In Vitro and In Vivo from 1.5 mm Beads of the 90L(+)/10G System Containing 10% WR-7557

between *in vitro* and *in vivo* release of WR-7557 for this system. Reevaluation of the candidate systems described above resulted in the initiation of both *in vitro* and *in vivo* testing using ^{35}S-labeled WR-7557; further testing with the Bratton and Marshall (1939) technique was discontinued. Samples of this 90L(+)/10G system were tested versus *P. berghei* challenge.

In Vivo Results of Selected Samples Using ^{35}S-Labeled WR-7557

Two materials were selected, based on the results of initial *in vitro* experiments summarized above, as candidates for a potential 90-day WR-7557 sustained release system. They were: 1.5 mm dia. spherical beads of the 50dl/50L(+) copolymer of approximately 150,000 MW containing 33.3% WR-7557 and 90 - 180μ cryogenically ground powder of the 100% L(+) system of approximately 250,000 MW containing 20% WR-7557. These two systems were also evaluated by WRAIR. In addition, a system of 0.75 mm dia. cylindrical rods of the 50dl/50L(+) system of approximately 150,000 MW containing 33.3% WR-7557 was selected for evaluation. Also, a test of 90 - 180μ powder ground from the 0.75 mm rods of the 50dl/50L(+) system containing 33.3% WR-7557 was initiated in an attempt to increase the rate of WR-7557 release from a matrix which had demonstrated very uniform, albeit slow, WR-7557 release *in vivo*.

The results of WR-7557 release from these matrices are presented in Figure 4 where the average cumulative percent ^{35}S-labeled materials excreted in the urine of mice is plotted as a function of time. Special note should be made here for the 90 - 180μ powder of the 100L(+) system. Initially, the 90 - 180μ powder of the 100L(+) system released over 18% of the initially injected WR-7557 in just four days. This was attributed to the release of WR-7557 readily accessible at the surface of the particles. The excreted release rate per week then dropped to less than 1% of the WR-7557 injected. Unfortunately, the 6 mg dose of matrix initially selected for administration proved too severe and the experiment ended when all four mice died. The experiment was repeated, at a dose of 4 mg of matrix, with the result that WR-7557 release was identical, on a percentage basis, to that of the 6 mg

dose, but without deleterious effects to the mice. It may be noted, however, that after one week in vivo, the 4 mg dose had released 20.3% of the WR-7557 injected while the 6 mg dose had released 20.0%; after two weeks, 21.8% compared with 21.5%; and after three weeks, 22.3% compared with 22.3%. Results of the tests with the 6 mg dose are presented in Figure 4.

The results presented in Figure 4 indicate that the initial release of WR-7557 from the polymer drug matrices may be a function of the external specific surface, or the ratio of the external surface area to the volume, of the preparations. The external specific surface, $S_{external}$, for the spheres is given by (where d = diameter):

$$S_{external} = \frac{6}{d} \frac{cm^2}{cm^3}$$

and for rods is given by:

$$S_{external} = \frac{4}{d} \frac{cm^2}{cm^3} .$$

Thus, for the 90 - 180μ spheres, using 100μ as the nominal diameter,

$$S_{external}\ (100\mu) = 600\ cm^2/cm^3;$$

for 0.75 mm dia. rods,

$$S_{external}\ (0.75\ mm) = 50.0\ cm^2/cm^3;$$

and for 1.5 mm dia. beads:

$$S_{external}\ (1.5\ mm) = 37.5\ cm^2/cm^3.$$

Thus, the ratio of WR-7557 released from materials of similar density and sample weight, based on the external specific surface for the 90 - 180μ powder: 0.75 mm dia. rods: 1.5 mm dia. beads would be 16:1.5:1.0. In the first three to four days following administration, it is shown in Figure 4 that the 90 - 180μ powders released in the urine 18.5% and 23.5% of the WR-7557

initially present in the sample, while the 0.75 mm dia. rods released 2.5%, and the 1.5 mm dia. beads released 1.5%. Taking the release from the 1.5 mm dia. beads as 1.0, this normalizes to a ratio of 12 - 16:1.7:1.0, nearly identical to that ratio predicted by comparing the external specific surfaces.

Once the initial surge in WR-7557 release was completed, as shown in Figure 4, all four preparations, regardless of size or shape, provided slow, uniform release of WR-7557 in the urine at rates of approximately 1 - 2%/week. In particular, the 0.75 mm dia. rods exhibited very uniform WR-7557 release for the entire 90 days. The exception was a slight burst in WR-7557 release from the 1.5 mm dia. beads of 5%/week in the middle of the release period. WR-7557 was excreted at a constant rate both before and after the burst, however.

This behavior, of rapid initial release followed by slow, apparently zero-order release, may be explained by the results of visual observation of the sites of administration upon termination of the experiment. The rods and beads were enclosed in a thin film of a hard, clear material. This was not apparent in the mice administered the 90 - 180μ powders. This material made it difficult to dissolve the rods and beads in dioxane in order to measure residual WR-7557 at the site of administration. In addition, this material may have walled off the implants, interfering with drug delivery, and allowed all four preparations to release WR-7557 at approximately the same rate.

After 91 days <u>in vivo</u> in mice, the 90 - 180μ powder of the 100L(+) system had released 30% of the WR-7557 sample in the urine. Urinary release of WR-7557 (or metabolites) from the 50dl/50L(+) system was 21% from the 1.5 mm dia. beads, 11% from the 0.75 mm dia. rods, and 40% from the 90 - 180μ powder [50dl/50L(+)] in the three-month period. Residual WR-7557 at the administration sites was 45% for the 1.5 mm dia. beads and 57% for the 0.75 dia. rods for totals of 66% and 68% accounted for, respectively. This is somewhat low and may be attributed to difficulties encountered when the residues were dissolved in dioxane. As noted, the thin film around the implants made them difficult to dissolve, and some residual WR-7557 may have been lost. Several

fecal samples were also measured for ^{35}S activity to check the assumption that WR-7557 excretion was primarily in the urine. Fecal samples were collected for the first three days from the mice injected with the 90 - 180μ powder of the 50dl/50L(+) system; no ^{35}S activity was detected.

In Vitro Results

The average cumulative percent ^{35}S-labeled WR-7557 leached *in vitro* from the matrices discussed above is illustrated in Figure 5. The results are very similar to those obtained *in vivo*, although there does not appear to be the one-to-one correlation as determined previously. The 90 - 180μ powder (100L(+)/20% WR-7557) again released WR-7557 most rapidly: 32% in 76 days. This one powder system evaluated *in vitro* also exhibited an initial burst in WR-7557 release, but not of the same magnitude as noted *in vivo*. After the initial surge in WR-7557 release, again attributed to removal of WR-7557 from the surface of the particles, the release rate slowed significantly and maintained a fairly steady rate for the remainder of the release period. WR-7557 release from the 1.5 mm dia. beads and from 0.75 mm dia. rods was much slower than from the powder, apparently due to the reduced surface area of the beads and rods. After 76 days *in vitro*, 4% of the WR-7557 originally contained in the beads had been released at a very slow, uniform rate. In 87 days, the 90 - 180μ powder of the 100L(+) system released 34% of the WR-7557 initially present in the sample; 6% in the first two days and the remaining 28% in the last 85 days. The 0.75 mm dia. rods and the 1.5 mm dia. beads of the 50dl/50L(+) system behaved similarly, releasing 4% and 5%, respectively, at a very slow, uniform rate. The residues remaining in the extraction thimbles contained 45%, 72%, and 79% of the WR-7557 initially present in the samples for totals of 79%, 76%, and 84% accounted for, respectively. This appears to be reasonable closure of the WR-7557 material balance, or inventory, indicating that the *in vitro* release data are significant and represent the pattern of WR-7557 release from these matrices.

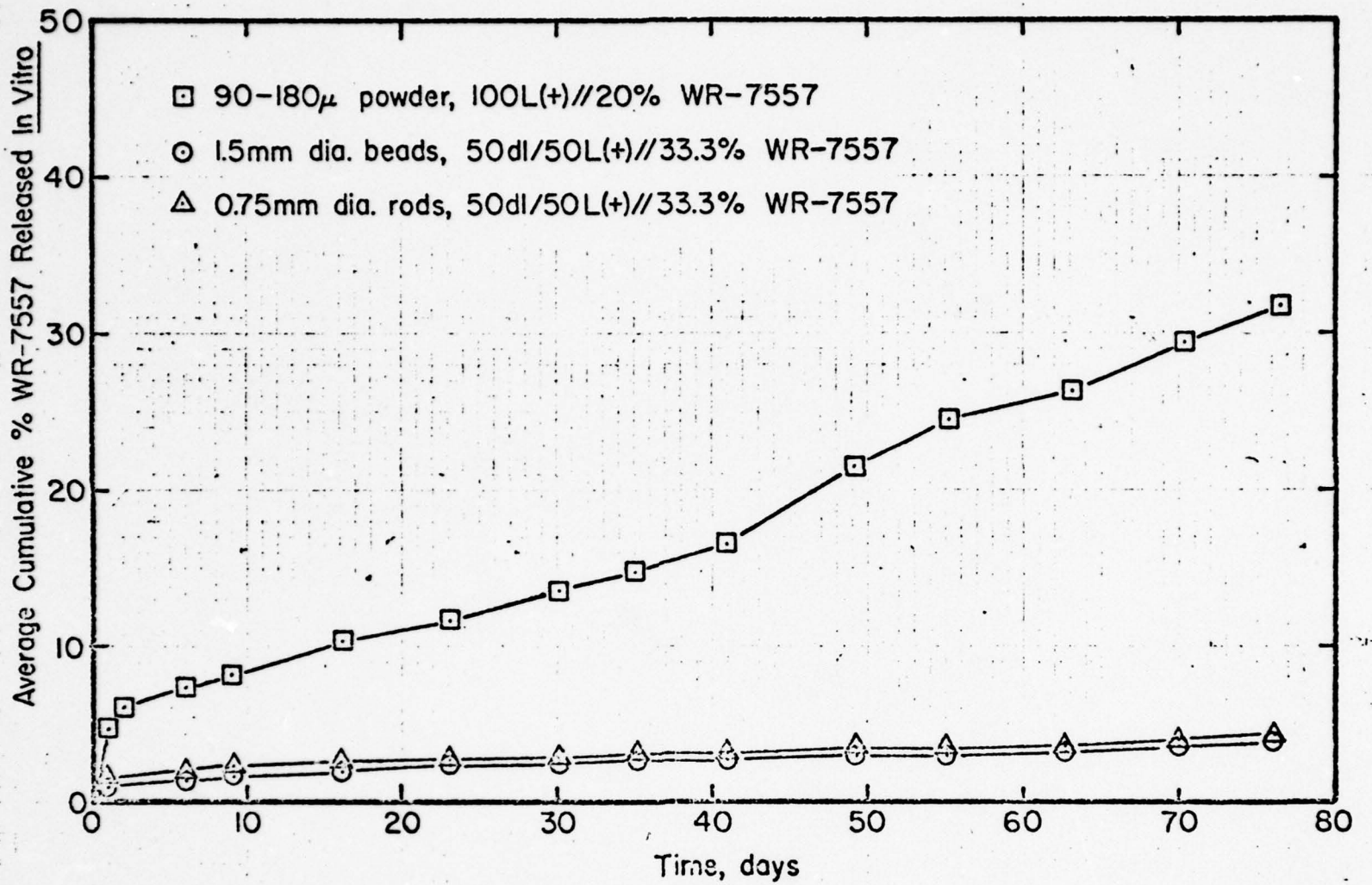

Figure 5

Average Cumulative Percent WR-7557 Released In Vitro from Polymer Matrices Containing ^{35}S-Labeled WR-7557

The first *in vivo* release rate data of ^{35}S-labeled WR-7557 from drug/polymer matrices indicated much slower WR-7557 excretion in the urine than anticipated; accordingly, the solubility of pure ^{35}S-labeled WR-7557 was measured *in vitro* in duplicate studies. An excess of WR-7557 was placed between two pieces of Millipore® 0.025μ filter, sealed with silicone rubber, and leached in 65 ml of pH 7 phosphate buffer at 37°C. Samples were taken periodically without changing the leaching solutions. The solubility of ^{35}S-labeled WR-7557 determined in this manner was approximately 610 μg/ml in pH 7 buffer at 37°C. This is of the same order of magnitude as the value of 960 μg/ml recorded at an earlier time in this laboratory.

Results of *P. berghei* Challenge Testing

A summary of the scope of the *P. berghei* challenge tests and the results are shown in Table 1; in Table 2 is presented results showing the week of first patency for the system tested. The detailed results of the *in vivo* testing in mice for drug efficacy and duration are shown in Figure 6.

The preparation of 1.5 mm dia. beads of 90L(+)/10G copolymer with 10.0% WR-7557 delayed patency of parasitemia to two and three weeks at doses of 66 and 132 mg/kg (four and eight beads) and had antimalarial activity through 20 weeks of infective challenging. No external evidence was seen of rejection (site-localized ulceration, loss of hair, and/or sloughing of skin) during the entire period of study.

The preparation of 1.5 mm dia. beads of 50dl/50L(+) copolymer with 33.3% WR-7557 delayed patency to three weeks at the lowest dose (57 mg/kg; one bead) and was completely effective at the higher doses. Two anomalous deaths occurred in the 115 mg/kg group. There was no external evidence of rejection observed during the entire period of study. At autopsies of three mice at 18, 23, and 24 weeks, respectively, the implanted beads were localized subcutaneously in sacs which also contained blood (by appearance). At autopsies of seven mice at 27 to 52 weeks, the implanted beads were intact in sacs without blood or evidence of local reaction.

Table 1

Summary Results of *Plasmodium berghei* Challenge Testing

System	Polymer Composition	Dosage Form	% by Wt. WR-7557 in Preparation	Range of Doses Studied In Vivo mg drug / kg body wt.	Efficacy	Biocompatibility
A	90L(+)/10G	1.5 mm dia. beads	10	17 - 132 (1 - 8 beads)	At doses of 66 and 132 mg/kg (4 and 8 beads) patency was delayed 1 - 2 weeks and there was prolonged survival.	No evidence of rejection.
B	50dl/50L(+)	1.5 mm dia. beads	33	57 - 456 (1 - 8 beads)	At all doses patency was delayed or absent and there were no deaths related related to malaria.	No evidence of rejection during period of study. At autopsies of 3 mice at 18, 23, and 24 weeks, respectively, the beads were localized in sacs which also contained blood by appearance. At autopsies of 7 mice at periods of 27 to 52 weeks, beads were intact and there was no evidence of local reaction.
C	100L(+)	90 - 180 μ powder	20	10 - 160	At 80 or 160 mg/kg there was a 1-week delay in patency.	No evidence of rejection.

Table 2

Results Showing Week of First Patency in Plasmodium berghei Challenge Experiments

SYSTEM	A			B			C			
Polymer Type	90L(+)/10G			50dl/50L(+)			100L(+)			
Dosage Form	1.5 mm dia. beads			1.5 mm dia. beads			90-180 powder			
% by Wt. Sulfadiazine (WR-7557)	10.0			33.3			20.0			
	# Beads	mg/kg	Week	# Beads	mg/kg	Week	Study #1 mg/kg	Week	Study #2 mg/kg	Week
	1	17	1	1	57	3	10	1		
	2	33	1	2	114	> 18	20	1	20	1
	4	66	2	4	228	> 18	40	1	40	1
	8	132	3	3	456	> 18	80	2	80	1
									160	2

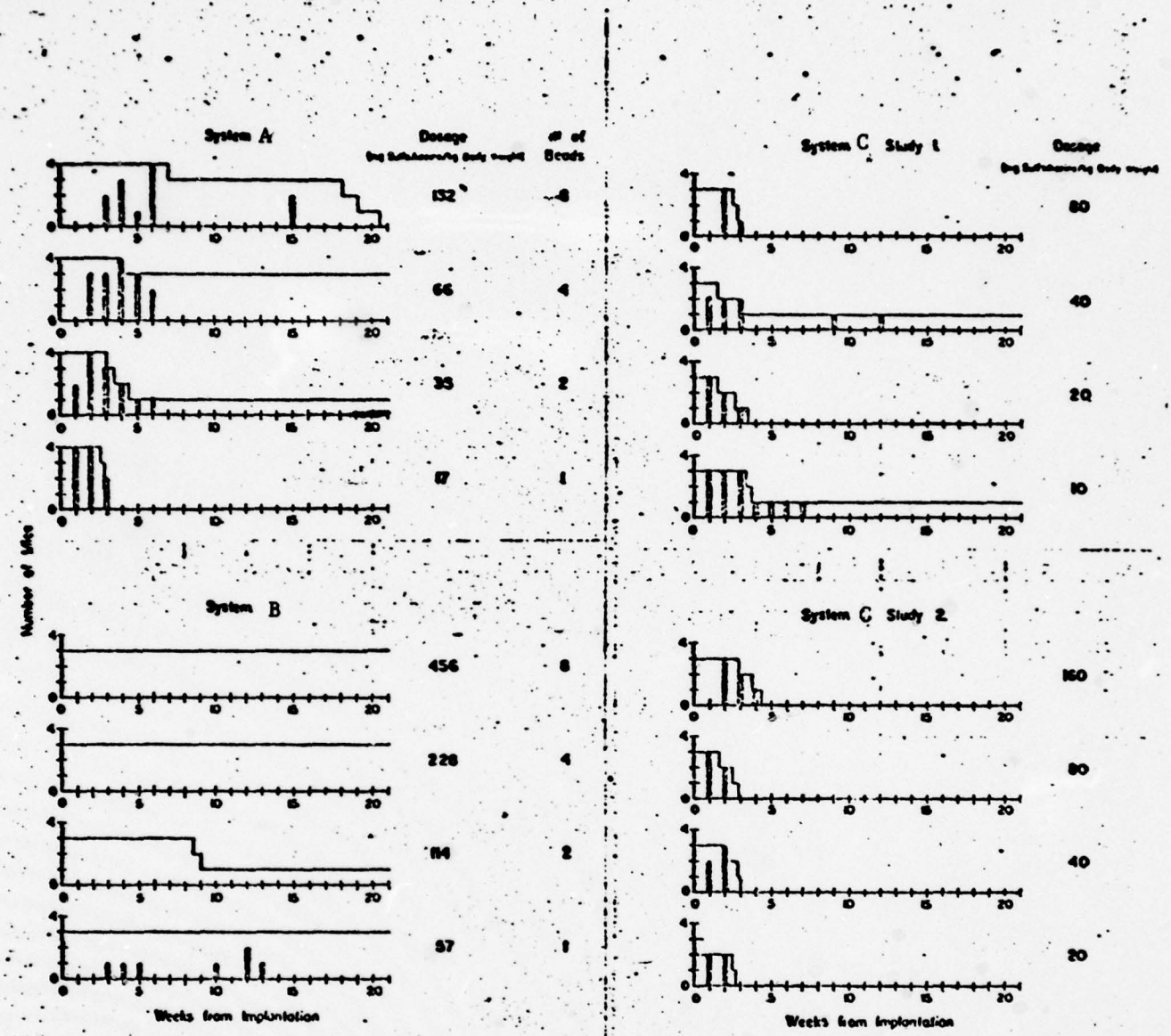

FIG. 6

[illegible]acy to Plasmodium berghei challenge of the three sulfadiazine (WR-7557) preparations at selected dosage levels. Plotted versus time (weeks from implantation) is the number of animals surviving (—) and the number of animals having positive blood smears (▯). The various systems are as follows: System A - 1.5 mm dia. spherical beads of the 90L(+)/10G copolymer with 10.0% WR-7557. System B - 1.5 mm dia. spherical beads of the 50dl/50L(+) copolymer with 33.3% WR-7557. System C - 90-180 μ powder of the 100L(+) polymer with 20.0% WR-7557 (the same preparation was used for Study 1 and Study 2 but over a different range of dosage levels).

The preparation of 90 - 180μ powder of 100L(+) polymer with 20.0% WR-7557 delayed patency to two weeks at 80 mg/kg in one study and 160 mg/kg in the second. At the second week, all surviving mice were malarious. Only two mice survived beyond five weeks. There was no external evidence of rejection observed nor was there evidence of local reaction observed at autopsy.

All control animals were malarious at the first week and subsequently died.

CONCLUSIONS

The results show clearly that subdermal implantation of the drug/polymer preparations was a successful procedure for delivery of sulfadiazine (WR-7557). The systems were efficacious against P. berghei; with the preparation of 1.5 mm dia. beads of 50dl/50L(+) copolymer with 33.3% WR-7557, there was complete protection at doses of 114 mg/kg and greater. The other preparations were not as successful, but this may be attributable in part to the generally lower ranges of dosage employed. The in vivo and in vitro tests using ^{35}S-labeled WR-7557 were largely supportive of the P. berghei challenge testing. In all cases, evidence of bio-incompatibility was generally negligible.

Based upon the results of this and the previous study (Wise, McCormick, et al, 1976), there are excellent prospects for development of a system for human application. The benefits to be derived are convenience and control. There would be a single administration without repetitive requirements and (when the implant is of an intact configuration) an easy removal if desired in case of adverse reaction or when exposure to malaria is terminated. The proper dose would be administered and would be present for a predetermined period of time. There would be no subsequently scheduled administrations with problems such as avoidance by the individual because of schedule conflicts or occasional shortages in supply of the drug. If an application may be successfully extended to humans, such a system is of potential prophylactic value in malarious areas for both the indigenous populations and military forces temporarily present by operational necessity (Canfield, 1972).

ACKNOWLEDGEMENT

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Appendix C

SUSTAINED RELEASE OF A DUAL ANTIMALARIAL SYSTEM

D. L. Wise, J. D. Gresser, and G. J. McCormick

SUSTAINED RELEASE OF A DUAL ANTIMALARIAL SYSTEM

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ABSTRACT

The sustained release of a dual drug system was evaluated in Rhesus monkeys and mice using a biodegradable carrier. The two drugs were ^{3}H-labeled sulfadiazine (WR-7557) and ^{14}C-labeled 2,4-diamino-6-(2-naphthyl-sulfonyl)-quinazoline (WR-158122). The polymeric carrier for each drug was synthesized from 90 parts by weight L-lactide and 10 parts by weight glycolide to a molecular weight of 46,000 ($\overline{M}w$). The dual system injected was a blend of two independent systems, each consisting of 50%(w/w) of the appropriate drug, in a weight ratio of ten parts of the WR-7557 system to one part of the WR-158122 system. This blend of finely divided cryogenically ground particles was injected intramuscularly into three Rhesus monkeys (macaca mulatta); a fourth monkey, serving as a control, received an equivalent dose of a ten-to-one ratio of combined pure drugs. Similar testing was carried out in mice. In monkeys, ^{14}C-labeled material derived from WR-158122 was found to be excreted at a uniform rate; carbon-14 from the pure drug mix was excreted at approximately 50 µg/day, calculated as WR-158122, from week three to week 13 while the drug polymer matrix released the drug at an essentially zero order rate of approximately 2.6 µg/day during this period. Release of tritiated materials derived from WR-7557 was approximately 82% of the injected dose in three weeks; only a slight difference in release characteristics between the pure drug mix and mixed drug/polymer matrices was observed. Results in studies with mice were generally consistent with the results in monkeys. The distinguishing feature between the two drugs, apparently accounting for the wide difference in excretion rates, appeared to be drug solubility (610 µg/ml for WR-7557, <1 µg/ml for WR-158122; measured in pH 7 phosphate buffer).

INTRODUCTION

The unreliability of self-administration of prophylactic antimalarial drugs is well recognized (1). A sustained release drug delivery system may minimize individual responsibility for self-medication. Recently, results were presented (2, 3) on the sustained release of the antimalarial drugs sulfadiazine (WR-7557) and 2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline (WR-158122). For WR-158122, testing in mice of a finely divided (<125 microns) spray dried matrix of 75/25 weight percent dl-lactide/glycolide copolymer resulted in antimalarial activity through 14 weeks (2). For WR-7557, testing in mice of an array of polymers and dosage forms demonstrated a wide range of release rates (3). With this background of extended drug release, the tests reported here were then conducted to evaluate a second goal in the administration of antimalarials, namely, simultaneous administration of more than one drug. A third goal will be to ascertain if synergism occurs with a multiple drug sustained release system. In the present tests, monkeys were used as the principal animal species in order to increase the size of the depot. Supporting tests were conducted in mice.

MATERIALS AND METHODS

Polymer Preparation

Glycolide and L-lactide, prepared from glycolic and L(+)-lactic acids, were polymerized following earlier reported procedures (2, 3). One copolymer of 90 parts by weight L-lactide and 10 parts by weight glycolide, synthesized to a molecular weight ($\overline{M}w$) of 46,000 (designated 90L(+)/10G), was used for all tests reported here.

Antimalarial Drugs

Sulfadiazine (WR-7557) was supplied by the Division of Medicinal Chemistry, Walter Reed Army Institute of Research (WRAIR). Tritiated

sulfadiazine (prepared by New England Nuclear) was added in trace quantity. 2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline (WR-158122) and this drug radiolabeled with ^{14}C in the 2-position of the quinazoline ring for use as a tracer were also supplied by WRAIR.

The solubilities of WR-7557 and WR-158122 were measured by placing excess amounts of each drug between two pieces of Millipore® 0.025μ filter, sealed with silicone rubber, and leached in 65 ml of pH^7 phosphate buffer at 37°C. Samples were taken periodically, without changing the leaching solutions, and the radioactivity of the samples determined by liquid scintillation. The solubilities of the two drugs determined in this manner were approximately 610 μg/ml for WR-7557 and <1 μg/ml for WR-158122, each determined independently in pH^7 buffer at 37°C.

Sample Preparation

Two samples of the polymer were weighed and then solvent-blended (benzene) with each of the two antimalarial drugs in amounts to produce a system containing 50% drug/50% polymer by weight. Films were case, solvent was removed, and the two sets of polymer/drug extruded separately into 0.8 mm diameter cylinders. The two sets of extruded cylinders were then independently cryogenically ground, sieved, and 90 - 180μ sized particles were retained. The two matrices were characterized by the following parameters:

50 wt % WR-7557//90L(+)/10G//46,000 mol. wt.
50 wt % WR-158122//90L(+)/10G//46,000 mol. wt.

The two matrices were then mixed in a conical rotating blender in a weight ratio of ten parts of the WR-7557 matrix to one part of the WR-158122 matrix; the resulting mixture was used for experimental injections. A second sample, for injection as a control, consisted of the two drugs (no polymer) blended in a weight ratio of ten parts of WR-7557 to one of WR-158122. Prior to injection into animals, samples of both systems (mixed matrix and mixed pure drug) were dissolved in dioxane for determination of activities by liquid scintillation counting. These activities are presented in Table 1.

Table 1

Activities of Drugs and Matrices Used in Sustained Release Experiments With Monkeys and Mice

Sample Description	Specific Activity, μCi/g
^{14}C-WR-158122 (pure)	145.3
^{3}H-WR-7557 (pure)	399.5
50 wt % ^{3}H-WR-7557 matrix	207.1
50 wt % ^{14}C-WR-158122 matrix	65.6
10/1 Mixture of WR-7557 and WR-158122 matrices	
Tritium Activity	186.1
Carbon-14 Activity	5.72

Monkey Studies

Four monkeys (Macaca mulatta*) of either sex and weighing between three and five kilograms were placed in individual cages equipped with automatic watering systems and center-drain waste collectors. The animals were fed once daily and had water ad libitum. Complete urine and fecal collections

were performed daily throughout the study. Three of the monkeys received the dual drug/polymer matrix and one received the dual drug admixture alone.

Drug Preparation and Administration

Three vials, each containing 800 mg of the WR-7557/WR-158122 polymer formulation and one vial containing 400 mg of the antimalarial admixture alone (without polymer), were prepared. The particle size of the pure drug mix averaged about 10 microns. For injection, each sample was suspended in 1% w/w methocel to a total volume of 4 ml. Particle suspension was achieved by a Vortex® laboratory mixer. The volume of injection was 0.5 ml/kg body weight which was equivalent to 50 mg/kg of the active component mixture (4.5 mg/kg WR-158122 and 45.5 mg/kg WR-7557). The injections were made through an 18 gauge needle into the hamstring muscle of the thigh. Following injection, the syringe, needle, residual drug and leakage from the injection site recovered with cotton swabs, were rinsed with a measured volume (about 100 ml) of scintillation grade dioxane. An aliquot of the rinse from each sample was analyzed for residual radioactivity.

Urine and Fecal Collections

Urine was collected daily for one week prior to injection and for two weeks following drug administration. The collector pan was rinsed with a small amount of tap water and the rinse was combined with the urine; the total volume of rinse and urine was recorded. For the first two weeks post injection, about 2 ounces of each daily collection were frozen for later analysis. After two weeks, urinary output was pooled on a weekly basis by combining ten percent of each day's collection for seven days. One ounce of this pool was then frozen. This was continued for three months following injection. Feces were collected daily for one week prior to and for two weeks after drug administration. Following this, feces were pooled weekly for three months. Daily fecal collections and weekly pools were homogenized. All samples were shipped frozen over dry ice. Combustion analysis of feces and scintillation analysis of urine were by conventional techniques as practiced earlier (2, 3).

Plasma Levels

Heparinized blood was drawn from the monkeys at different times following injection. The plasma was separated and assayed for non-volatile ^{3}H and ^{14}C activity by air-drying single 1, 2 or 3 ml samples overnight, followed by combustion analysis.

Mouse Studies

Tests were done essentially following the procedures above. Two groups (5 mice/group) of CD-1 mice (Charles River Breeding Laboratories) weighing approximately 20 g each were given subcutaneous injections of the mixed matrix and the pure mixed drugs as indicated in Table 2. These weights delivered doses of 113.6 mg/kg of WR-7557 and 11.4 mg/kg of WR-158122.

Table 2

Injection Information for Mice

Sample Description	Wt. Injected/Mouse*
Mixed Drug/Polymer Matrix	5.0 mg
Mixed Pure Drugs	2.5 mg

*Matrix and mixed drugs were suspended in 1% methocel.

RESULTS AND DISCUSSION

Mouse Tests: WR-7557 (^{3}H) Release

Figure 1 presents excretion of tritium-labeled materials derived from sulfadiazine (WR-7557) in the mixed drug/polymer matrix as well as excretion derived from the pure drug mixture. Both preparations released

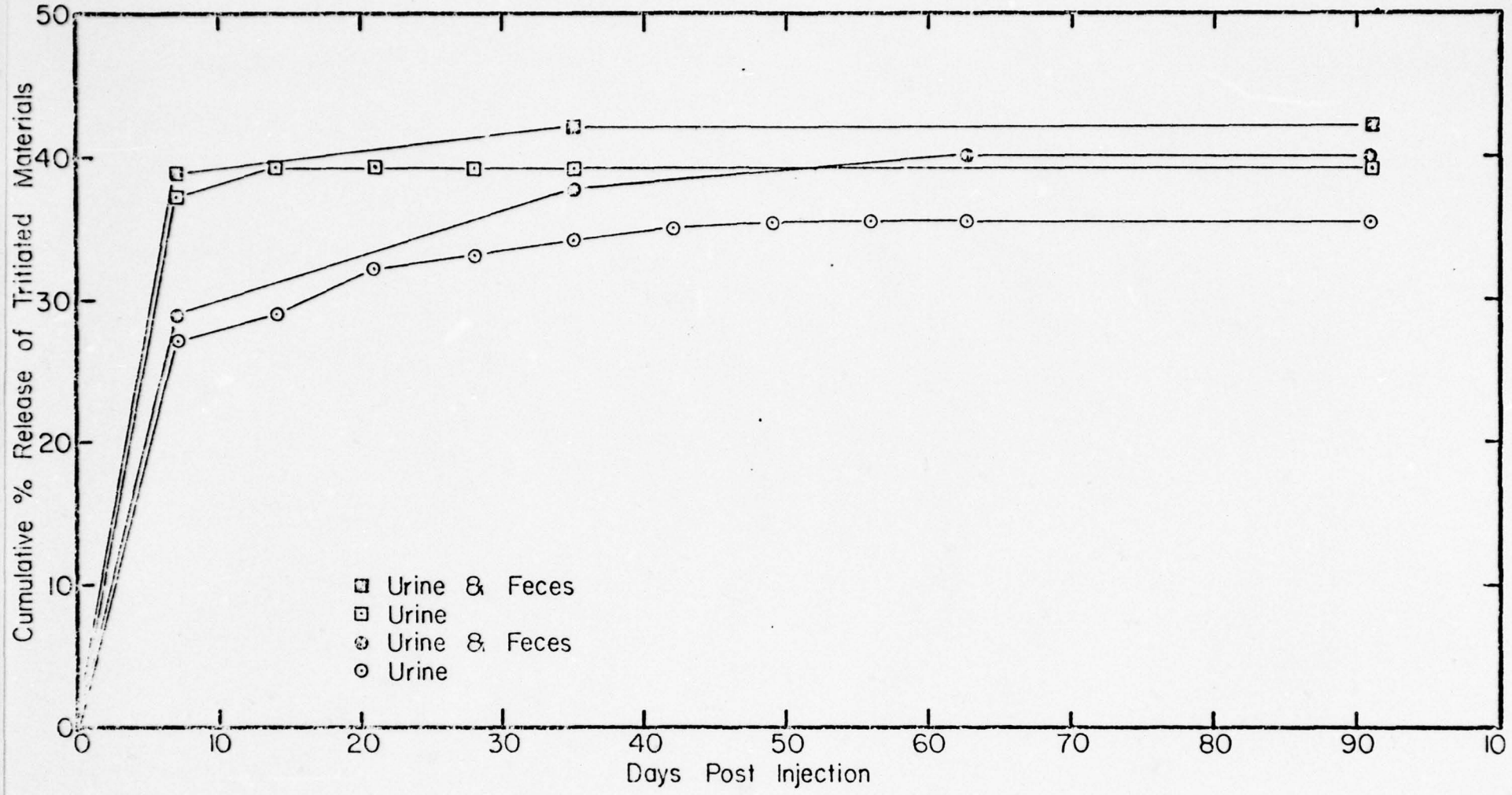

Figure 1. Release of WR-7557 as ^{3}H-Material Expressed as Cumulative Percent of the Initial Dose of ^{3}H (WR-7557) in Mice

Circle: 50 wt% Dual Drug Matrix (10/1 WR-7557/WR-158122)
Square: Pure Drug Mixture (10/1 WR-7557/WR-158122)

considerable amounts of sulfadiazine in the first week post injection; 39% of the initial dose of the pure drug mixture and 29% from the mixed matrix. The mixed matrix continued to release until week nine, although at a much reduced rate; from week two through five about 9% was released and another 3% from week six through nine. The pure drug mixture released only about 3% between weeks two through five, after which no sulfadiazine release was observed. No tritium was detected in urine of animals given the pure drug mix from week three to the end of the experiment, whereas tritium was present in diminishing quantities until week seven in urine of mice receiving the matrix. The total amounts released by week 13, when the experiment was terminated, were 42.3% (pure drug mixture) and 40.2% (mixed matrix).

Mouse Tests: WR-158122 (^{14}C) Release

Release of WR-158122 from mixed matrix and pure drug mix samples is shown in Figure 2. Urinary excretion patterns are quite similar and the 13-week recovery was approximately 26% in both cases. At the end of the first week the pure drug system had released 9.3% as compared with 5.1% for the mixed matrix. Urinary excretion curves indicate that about the same percent difference was maintained until week nine whereupon the curves began to converge. Total (urinary plus fecal) excretion of carbon-14 indicated a significant retarding effect of the polymer. After one week pure dual drug sample had released 21% as compared with 10.5% released by the dual drug matrix sample. After 13 weeks the former had released 80%; the latter only 50% of the initial radioactivity. During the latter weeks of the experiment (weeks 9-13), the release rate from the mixed pure drug sample had dropped to 0.8% per week whereas the mixed matrix was still releasing ^{14}C labeled materials at a rate of 1.4% per week.

Monkey Tests: Injection Data

Three Rhesus monkeys (macaca mulatta) were given intramuscular injections of the dual drug matrix and one monkey, serving as a control, was

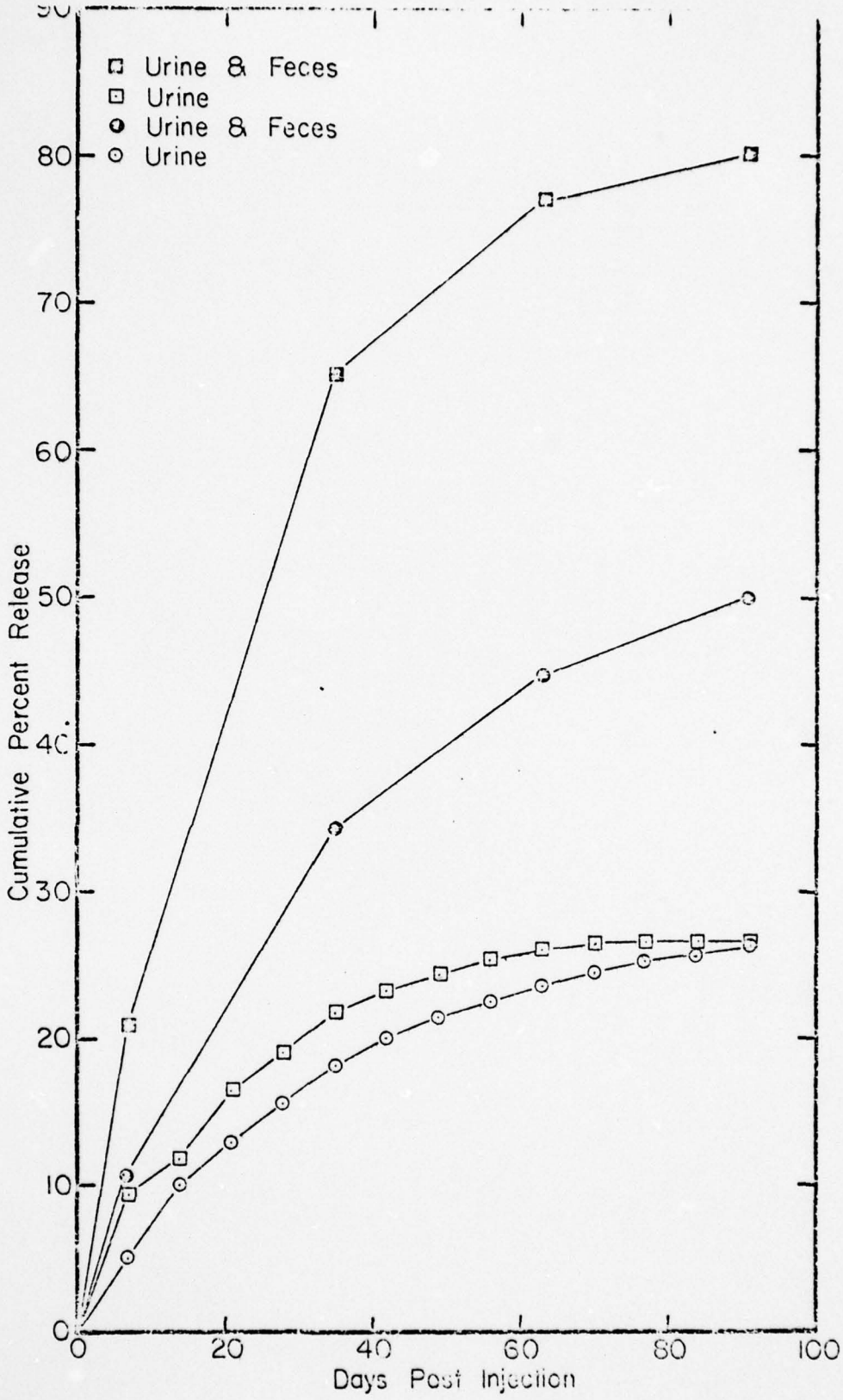

Figure 2. Release of WR-158122 as ^{14}C-Materials Expressed as Cumulative Percent of Initial Dose of ^{14}C (WR-158122) in Mice

Circle: 50 wt% Dual Drug Matrix (10/1 WR-7557/WR-158122)
Square: Pure Drug Mixture (10/1 WR-7557/WR-158122)

given an equivalent dose of pure dual drugs. A summary of injection data is given in Table 3. Quantities injected were determined as the differences between the quantities submitted and the residues remaining in the syringes and delivery vials after injection, as determined by liquid scintillation counting. In Table 3 are presented microcuries of ^{3}H and ^{14}C injected and grams injected as calculated from the activity. In all cases but one the weights injected based on ^{3}H are greater than those based on ^{14}C by factors ranging from 1.28 to 1.39, which may reflect possible inaccuracy in the initial determination of specific activity. In the case of monkey 6778 the quantity calculated on the basis of tritium was 3.26 times greater than that based on carbon-14. Although there is no obvious explanation for this discrepancy, it may be related to a leak-back of material from the injection site which occurred with this animal. Also in Table 3 are data on doses delivered to individual monkeys 6778, 6725 and 6724 (those receiving the mixed matrix). The average dose delivered to these animals was 85.8 mg/kg of matrix (drug + polymer) whereas the target dose was 100 mg/kg of the matrix. The low average is due to the leak-back experienced with monkey 6778. Monkey 6768, injected with the pure drug mixture, received a dose of 52.2 mg/kg which was very close to the target dose of 50 mg/kg. Note that these data in Table 3 are based on values computed as averages from ^{3}H and ^{14}C measurements of residues remaining after injection.

Monkey Tests: WR-7557 (^{3}H) Release

Tritium excretion by all monkeys was virtually complete after three weeks. All monkeys had excreted between 74.0 and 93.5% of injected tritium radioactivity by the completion of the 13 week experiment. As illustrated in Figure 3, the presence of polymer did not significantly retard the release of tritiated materials derived from sulfadiazine (WR-7557). The release from monkey 6787 which had received the pure drug mixture was not observably different from the others; by the end of the third week monkey number 6787 had excreted 77.5% as compared with an average of 80.5% for the other three. At

Table 3

Weights and Activities of Samples Injected in Monkeys

I.D. # (1)	Description of Dose	Weight grams	Micro-curies ^{14}C	Micro-curies ^{3}H	Residues from Injection of Rhesus Monkeys: Residue(1) DPM/ml	Total µCi in Residue	µCi Original Sample	µCi(2) Injected	Grams(3) Injected	% Variation = $\frac{\text{Dev}}{\text{Avg}} \times 100$	Monkey Wt. Kilograms	Average Wt. Injected grams	Dose mg/kg
					^{14}C DPM/ml ^{3}H DPM/ml	^{14}C ^{3}H	^{14}C ^{3}H	^{14}C ^{3}H	Based on ^{14}C Based on ^{3}H Average				
6787	Pure dual drug mixture	0.3984	5.2625	144.69	69116.7 1466594.6	3.12 66.06	5.26 144.69	2.14 78.63	0.162 0.217 0.190 ± 0.028	14.7	3.64	0.190	52.2
6778	Mixed matrix	0.8003	4.5777	148.94	85242.3 1565183.8	3.84 70.50	4.58 148.94	0.74 78.44	0.129 0.421 0.275 ± 0.146	53.1	4.10	0.275	67.1
6725	Mixed matrix	0.7827	4.4776	145.70	60096.8 1586757.8	2.70 71.48	4.48 145.68	1.78 74.20	0.311 0.399 0.333 ± 0.044	12.4	4.08	0.355	87.0
6724	Mixed matrix	0.7999	4.5754	148.86	50367.4 982334.8	2.27 44.25	4.58 148.86	2.31 104.61	0.404 0.562 0.483 ± 0.079	16.4	3.86	0.483	125.1
Avg. for #'s 6778, 6725, 6724 (mixed matrices)								1.61±0.58 3575±1257			4.01±0.10	0.371±0.005	93.07± 21.37

NOTES:

(1) All residues dissolved in 100 ml dioxane for liquid scintillation counting.

(2) µCi injected = µCi (submitted) - µCi (in residue).

(3) Conversion of µCi (^{14}C or ^{3}H) injected into grams of pure drug:

Ex. 6787 $\quad gm = (2.14\ \mu Ci\ ^{14}C)\left\{\frac{1\ g\ mixture}{13.209\ \mu Ci}\right\}(10^3\ mg/g)$

Ex. 6778 $\quad gm = (0.74\ \mu Ci\ ^{14}C)\left\{\frac{1\ g\ matrix}{5.72\ \mu Ci}\right\}(10^3\ mg/g)$

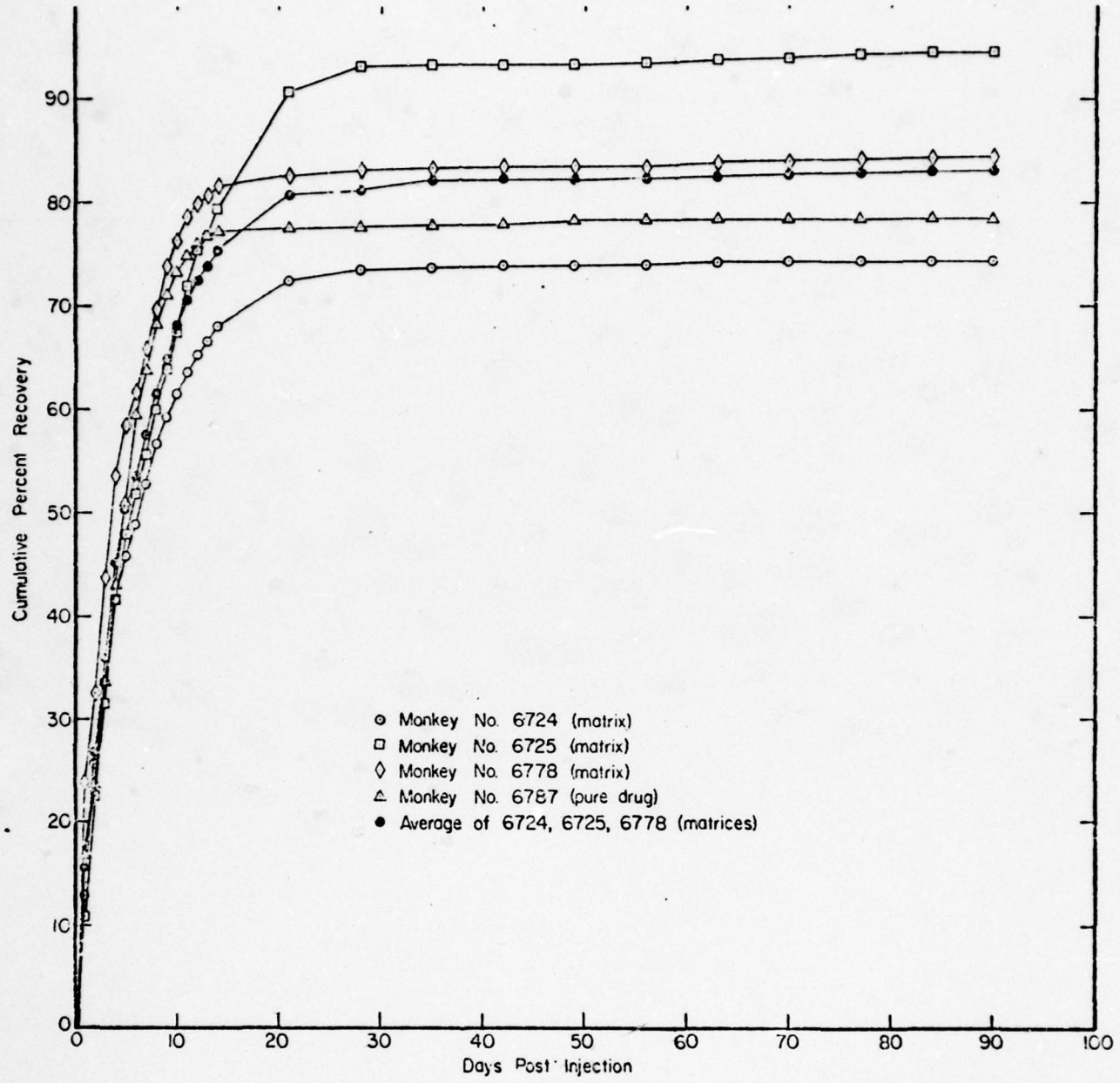

Figure 3. Release of WR-7557 ^{3}H-Material (total in Urine and Feces) Expressed as Cumulative Percent of Initial Dose of ^{3}H (WR-7557) in Rhesus Monkeys

50 wt% Dual Drug Matrix (10/1 WR-7557/WR-158122)
Pure Drug Mixture (10/1 WR-7557/WR-158122).

completion it had excreted 78.3% compared to the average 22.4% for those receiving the drug/polymer matrix. The injection data (Table 3) indicate that monkey number 6778 received only 0.74 μCi of ^{14}C-WR-158122, which is significantly lower than the quantities delivered to the others. This is most probably due to the leak back on injection. However, in spite of this, this monkey received a dose of ^{3}H-labeled sulfadiazine comparable to the others, with only number 6724 receiving more. In all cases except number 6778, the ratio of ^{3}H to ^{14}C activity lay between ∿37 and ∿45, which indicated satisfactory homogeneity between samples. Monkey number 6778 had a ^{3}H/^{14}C ratio of 106. As all sample vials supplied for testing were filled from the same batch of matrix, it is possible that the deviation of the ratio in the latter case may be associated with differential expulsion of the component matrices making up this sample at the leak back. Close agreement exists between the microcuries of ^{3}H excreted at various times for all animals. The three monkeys injected with the drug/polymer matrix released between 66.2 and 77.8 μCi ^{3}H total (urinary plus fecal excretion) in 13 weeks. Urinary excretion accounted for between 53.7 and 68.1 μCi of tritium. Comparable values at three weeks are 64.8 to 75.9 μCi for total excretion and between 52.6 and 66.6 μCi ^{3}H for urinary excretion. Data for monkey 6787 (pure drug mixture) fell within the range of values of the other animals: a total of 61.6 μCi ^{3}H were excreted in 13 weeks, of which 50.8 μCi ^{3}H were in the urine; at three weeks the monkey had excreted essentially all of the measured radioactivity. It is apparent that the low molecular weight polymer at 50% loading exerted little or no retarding effect on sulfadiazine release. There is a depot effect of approximately three weeks due to the solubility of sulfadiazine, not to the presence of the polymer.

Plasma levels of ^{3}H-WR-7557 are presented in Table 4; a semi-logarithmic plot of plasma levels vs. days post injection for the mean plasma levels of the three monkeys receiving the matrix and the one monkey receiving only the pure drug mixture is given in Figure 4. Maximum plasma levels were observed at the first sampling period, 6 hours post injection. These were very nearly the same for the matrix and the pure drug mixture (18.9 μg/ml and

Table 4

Plasma Levels of ^{3}H-Material in Rhesus Monkeys Following An Intramuscular Dose of 45.5 mg/kg WR-7557-^{3}H and 4.5 mg/kg WR-158122-^{14}C

Time Post Injection	Matrix System (Monkey Identification)				Pure Drug Mixture
	(6724)	(6725)	(6778)	Mean (3 Monkey)	(6787)
(Days)	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
	0.0	0.0	0.0	0.0	0.0
0.25	23.3	15.3	18.0	18.9	21.8
1	17.6	11.8	9.6	13.0	8.9
4	5.9	5.7	4.5	5.4	4.2
7	6.4	4.3	3.3	4.7	4.2
14	2.5	2.4	0.8	1.9	0.5
21	0.8	1.7	0.4	1.0	0.2 (1)
28	0.4	0.5	0.2(1)	0.4	0.1
35	0.3(1)	0.2	0.1	0.2 (1)	0.1
42	0.1	0.2(1)	0.1	0.1	0.1
49	0.1	0.1	0.0	0.1	0.0

(1) Limit of detection = 0.14 µg/ml plasma.

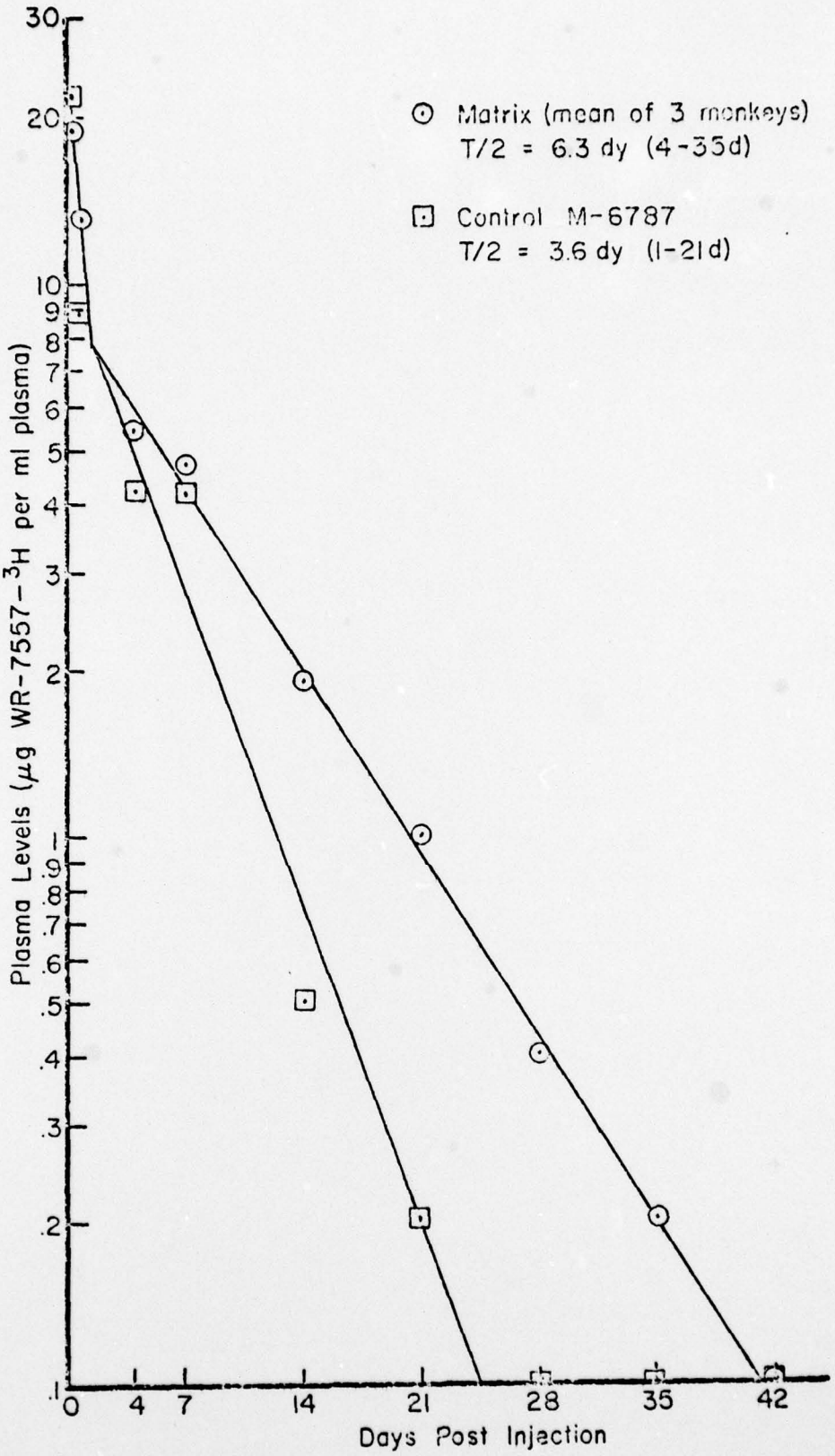

Figure 4. Plasma Levels of WR-7557-^{3}H in Rhesus Monkeys Following a 45.5 mg/kg Injection of WR-7557 and 4.5 mg/kg of WR-158122-^{14}C

21.8 µg/ml, respectively), indicating a similar rate of adsorption of the drug from the injection site. The plasma levels fell more rapidly with the pure drug mixture and the terminal half-life was determined to be 3.6 and 6.3 days for the two preparations, as shown in Figure 4. Over-all, the matrix system yielded higher plasma levels in the 1 to 6 week post-injection period, with plasma levels being 2 to 3-fold greater than the pure drug mixture. On the other hand, as is to be noted from Table 4, the plasma levels of the monkey receiving only drugs (6787) are comparable with the lowest of the three monkeys receiving the matrix (6778); it may be assumed that this comparison is due to the leak-back upon injection with monkey 6778.

Monkey Tests: WR-158122 (^{14}C) Release

As seen in Figure 5, at the completion of the experiment (13 weeks) release of ^{14}C-labeled materials in monkeys injected with the drug/polymer matrix varied from 0.36 to 0.47 µCi ^{14}C, of which 0.19 to 0.29 µCi ^{14}C had been excreted in urine. In terms of percentages, this represents a total excretion of about 19% of the initial dose for monkey number 6724 and 6725 and 64.5% for monkey number 6778. Apparently, the latter released as much radioactive material as the other two in this group, but this represents a larger fraction of the injected material because of loss due to the leak-back. Monkey 6787, recipient of the pure drug mixture, had excreted substantially more radioactivity at 13 weeks: 0.56 µCi ^{14}C in the urine, 1.0 µCi ^{14}C total (26.2% in the urine, 46.9% total). Monkey number 6778 also experienced a surge in urinary ^{14}C excretion during week 10 which accounted for 0.11 µCi or 14.4% of the injected ^{14}C activity. A normal excretion rate was resumed thereafter. Using the value of 145.3 µCi ^{14}C/g for the activity of pure WR-158122, rates of release of the drug were computed from the slopes of the plots of excretion versus time between days 21 and 90 (Figure 5). For monkey number 6787 this was 53.5 µg/day (as WR-158122) and an average of only 2.58 µg/day (as WR-158122) for the other three animals. The release of WR-158122 by the pure drug mixture injected into monkey number 6787 was relatively constant over the latter period

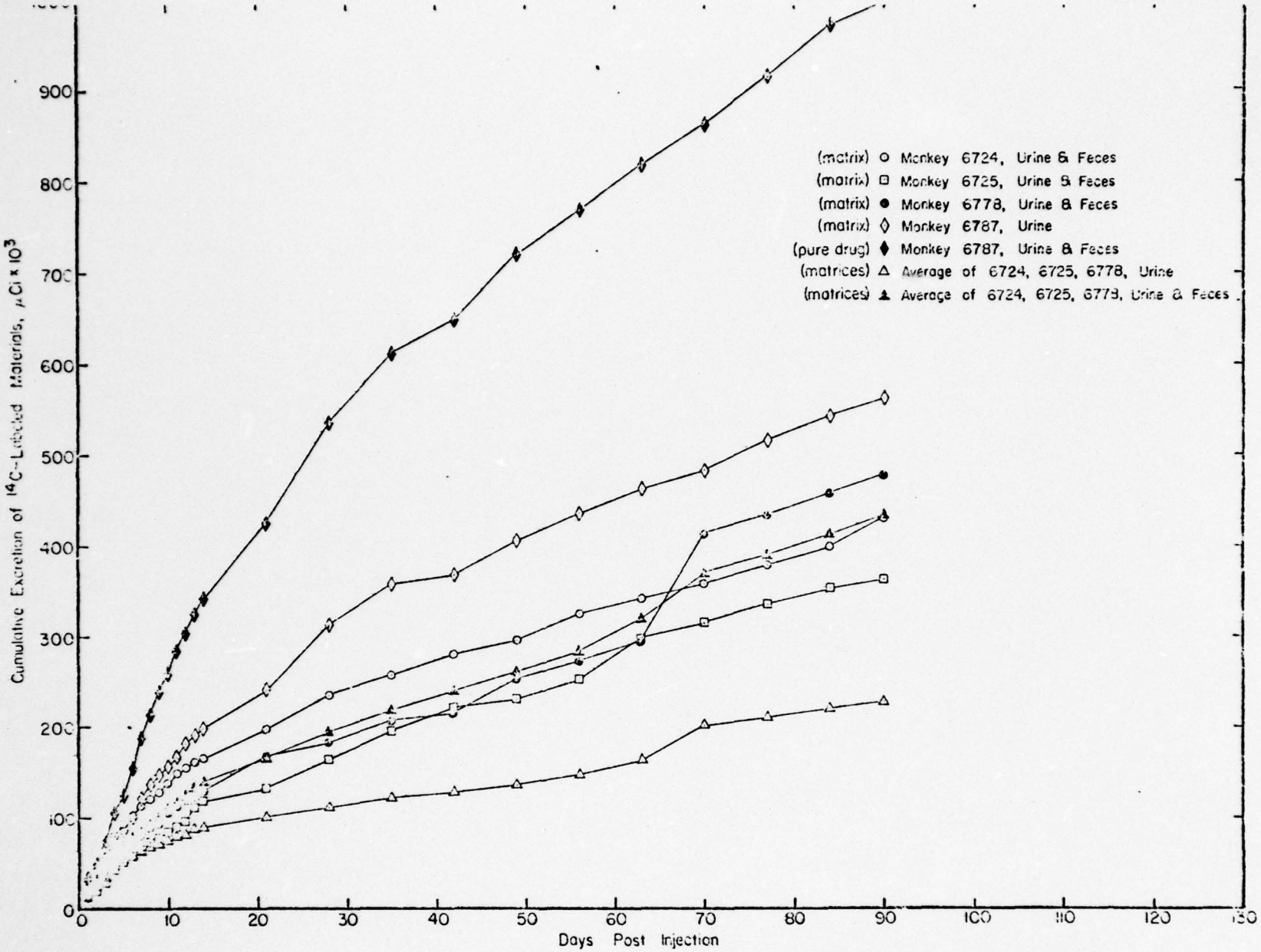

Figure 5. Release of WR-158122 in Rhesus Monkey Studies, Expressed as Microcuries of ^{14}C-Materials Excreted

50 wt% Mixed Drug (10/1 WR-7557/WR-158122) polymer matrix
Pure Drug Mixture (10/1 WR-7557/WR-158122)

of the study, averaging 47.5 µg/day (as WR-158122) between days 50 and 90. Daily excretion for this monkey, expressed as µCi ^{14}C and µg WR-158122, is plotted in Figure 6.

Plasma levels for WR-158122 are shown in Table 5, expressed as nanograms of drug-equivalents per ml of plasma. Assuming twice the background count as the limit of ^{14}C detection it was determined that none of the assay values were statistically significant. Specific activity of the ^{14}C-WR-158122 was too low to measure WR-158122 concentrations below 100 nanograms per ml with any degree of certainty. The data of Table 5, however, are of interest and are presented for completeness.

CONCLUSIONS

It is believed this is the first reported results on the sustained release of a dual drug system. Over-all, the results showed that the drug/polymer matrix provided for longer term release than the pure drug mixture. However, further significance may be attached to the substantial difference in release rates observed between the two drugs. Since the same polymer matrix, preparation, technique and dosage form were used in each case, these results may be attributed to the wide differences in drug solubilities. On this basis then, these studies may be viewed as that of a model system in which it was shown that design of this type of sustained release drug delivery system is strongly dependent upon drug solubility. Projections of the "lifetime" of a given sustained release system should therefore include drug solubility as a major design factor as well as polymer type, drug content in the matrix and system geometry.

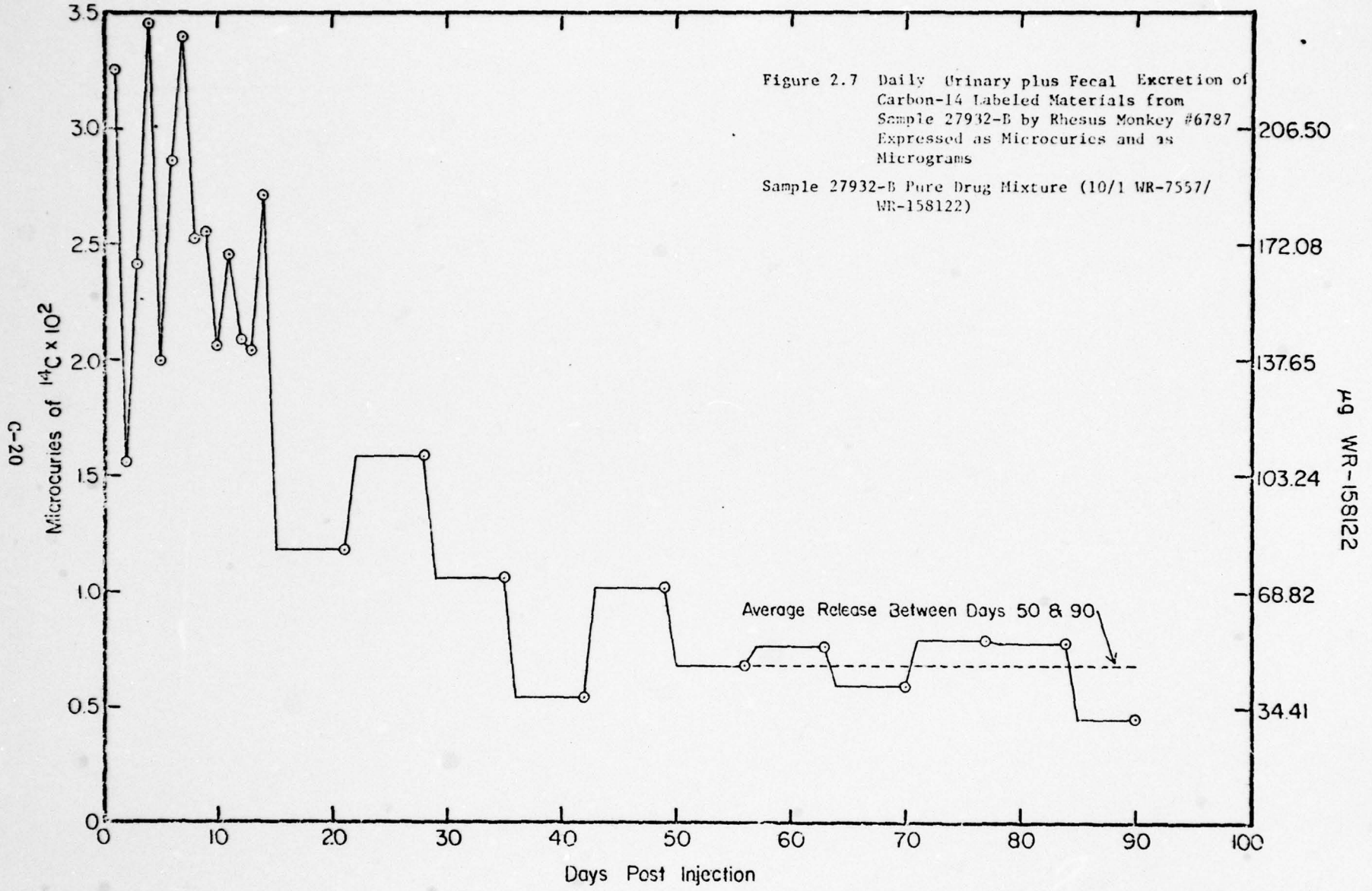

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Figure 6. Daily Excretion of ^{14}C, WR-158122 Derived Materials from Pure Drug Mixture (10/1 WR-7557/WR-158122) by Rhesus Monkey #6787, Expressed as Microcuries and as Micrograms

Table 5

Plasma Levels of ^{14}C-Material in Rhesus Monkeys Following an Intramuscular Injection of 45.5 mg/kg WR-7557-^{3}H and 4.5 mg/kg WR-158122-^{14}C

Time Post Injection	Matrix System (Monkey Identification)				Pure Drug Mixture
	(6724)	(6725)	(6778)	Mean (3 Monkey)	(6787)
(Days)	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
	0	0	0	0	0
0.25	37	28	34	33	56
1	39	11	23	24	62
4	23	11	15	16	39
7	23	7	11	14	49
14	15	7	12	11	41
21	15	7	11	11	34
28	11	8	15	11	26
35	19	15	11	15	26
42	19	11	8	13	24
49	19	15	15	16	22

Note: Limit of detection = 100 ng/ml plasma.
= 0.1 µg/ml plasma.

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Footnote

*In conducting the research described in this report, the investigators adhered to the principles set forth in the Guide for Care and Use of Laboratory Animals as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute for Laboratory Animal Resources, National Research Council - National Academy of Sciences.

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